Ion Transport. Pb has been shown to disrupt the transportation of critical cations across the cell membrane by decreasing the activity of ATPases (including Na+/K+-, Ca2+, and Mg2+-ATPases; reviewed by EPA 2014c). Pb-induced inhibition of ATPase activities has been shown in the kidneys, livers, erythrocytes, and brain synaptosomes of rats exposed to Pb in drinking water; in testes of rat pups exposed during lactation and postweaning; in primary cerebellar granule neuronal cultures of rat pups exposed pre- and postnatally; in rabbit kidney membranes and sarcoplasmic reticulum exposed *in vitro*; and in human erythrocyte ghosts. Furthermore, blood or hair Pb levels were inversely correlated with ATPase activities in erythrocytes in several human epidemiological studies.

In addition to ATPases, Pb's action on ion transport includes competitive inhibition of voltage-gated calcium channels (reviewed by EPA 2014c). A number of *in vitro* studies have demonstrated inhibition of calcium transport via voltage gated channels in cultured neurons and neuroblastoma cells, bovine adrenal chromaffin cells, and human embryonic kidney cells. Inhibition of calcium transportation via voltage-gated channels can disrupt release of neurotransmitters, and impaired neurotransmitter release has, in fact, been shown with Pb exposure at low *in vitro* levels. In addition to inhibiting calcium-dependent neurotransmitter release, Pb may mimic calcium, thereby increasing neurotransmitter release in some circumstances. For example, Pb exposure *in vitro* has been shown to induce the spontaneous release of norepinephrine from bovine adrenal chromaffin cells and increase the release of catecholamine from PC12 cells. It has been suggested that Pb may trigger spontaneous neurotransmitter release via activation of calcium/calmodulin-dependent protein kinase II-dependent phosphorylation of synapsin I, or by directly activating synaptotagmin I (a calcium-sensing protein that regulates neurotransmitter release). Intracellular migration of Pb has been shown to occur via calcium channels; higher Pb permeation in several cell lines (HEK293, HeLa, and PC12) correlated with lower calcium concentrations, suggesting that Pb competed with calcium for the channel binding sites.

Pb also disrupts the activity of calcium-dependent potassium channels, as shown by increased efflux of potassium from inverted erythrocyte vesicles, and alterations in potassium channel activation in erythrocytes exposed to Pb (reviewed by EPA 2014c). The nature of the effect on potassium channels is dose-dependent; at low Pb concentrations (<10 μM), potassium channels are activated, while inhibition of the channels is seen at higher Pb concentrations. As with calcium channels, alterations in potassium channel activity may also disrupt neurotransmitter release. In rats exposed to Pb *in utero* and postnatally, potassium-stimulated release of hippocampal GABA was decreased at low exposure levels, but enhanced GABA release was observed at higher exposures (in the absence of calcium).

Cellular Energetics. Evidence indicating that Pb exposure perturbs mitochondrial function and cellular energy metabolism is abundant (as reviewed by EPA 2014c). In rats exposed to Pb via diet or drinking water, renal tubular and epididymal mitochondria exhibited swelling, rupture of the outer membrane, distorted cristae or loss of cristae, vacuolization, inclusion bodies, and fusion with nearby mitochondria. As discussed further in Section 2.21.6, Apoptosis, Pb exposure has been shown to open the mitochondrial transmembrane pore, initiating the apoptotic caspase cascade. Evidence for Pb's effect on energy metabolism includes decreased ATP levels and/or adenylate energy charge (AEC) (along with increased ADP, AMP, and/or adenosine levels) in forebrain synaptosomes from rats exposed via drinking water, in cerebellar granule neuronal cultures from rats exposed by drinking water, in PC-12 cells exposed *in vitro*, and in isolated mitochondria exposed *in vitro*. In osteoblasts exposed *in vitro*, Pb inhibited both coupled and uncoupled respiratory oxygen use in mitochondria. Pb has been proposed to behave as a classic chemical uncoupler of respiration, abolishing the proton gradient necessary for oxidative phosphorylation. In the muscles of rats exposed to Pb in drinking water, decreased activities of the enzymes of complex I and IV of the respiratory chain were observed. However, in forebrain synaptosomes from rats exposed to Pb *in vivo*, oxidative phosphorylation was not inhibited, despite the fact that ATP levels were decreased.

Pb may affect cellular energetics via perturbation of the glycolysis pathway. Decreased glycolysis was observed in osteoblasts and erythrocytes exposed to Pb *in vitro* (reviewed by EPA 2014c). However, increased levels of glycolytic enzymes were noted in workers with higher blood Pb levels, when compared with workers with lower blood Pb, suggesting that Pb may activate anaerobic glycolysis.

Depletion of cellular nucleotide pools required for ATP synthesis has also been observed after Pb exposure of human erythrocytes *in vitro* and in rats exposed via drinking water (reviewed by EPA 2014c). This effect may be mediated by Pb-induced inhibition of enzymes involved in nucleotide biosynthesis in erythrocytes, including adenine phosphoribosyltransferase (see Impaired Protein Function below) and NAD synthetase (which depends on magnesium for activity). In support of the latter mechanism, in humans exposed to Pb, PbB levels were inversely correlated with NAD synthetase activity.

Impaired Protein Function. Pb impairs the functions of numerous proteins, with concomitant effects on signaling, growth and differentiation, gene expression, energy metabolism, and biosynthetic pathways. The mechanisms by which Pb alters protein activity are by displacing metal cofactors or binding to sulfhydryl groups (reviewed by EPA 2014c). Table 2-49 shows proteins known to be bound to or

Table 2-49. Effects of Lead (Pb) on Function of Various Proteins

Protein	General function	Effect of Pb; summary of evidence
Calcium-dependent proteins		
Calcium binding proteins (CABPs I and II)	Regulation of calcium signaling, especially in neuronal cells	No data -Ca^{2+} displacement shown *in vitro.*
Ca^{2+}-dependent K^+ channel	Ion transport; activation of channels regulates neuron firing and neurotransmitter release	Activates or inhibits channel -Pb promoted efflux of K^+ from inverted red blood cell vesicles. -Pb induced activation of K^+ channel in erythrocytes at low Pb concentrations and inhibited activity at high concentrations.
Calmodulin	Cell signaling, including structural integrity, gene expression, and maintenance of membrane potential	Amplifies calmodulin activity -Pb activated calmodulin-dependent phosphodiesterase and cyclic nucleotide phosphodiesterase activities. -Pb stimulated brain membrane phosphorylation. -Pb increased binding of calmodulin to brain membranes.
Mitochondrial transmembrane pore (MTMP)	Triggers mitochondrial apoptosis cascade when open	Opens MTMP, triggering apoptosis -Pb increased mitochondria-regulated apoptotic indicators (cytochrome c, caspases) in rat retinal rod cells and hepatic oval cells *in vitro*.
NAD(P)H oxidase	Inflammatory mediator; triggers oxidative burst (via production of superoxide) in response to infection	Increases activity, leading to ROS generation -Pb increased protein levels of glycosylated subunit of NAD(P)H oxidase in brain, heart, and renal cortex of rats exposed via drinking water and in human coronary artery endothelial cells *in vitro*.
Osteocalcin	Bone resorption, osteoclast differentiation, and bone growth	Alters binding of osteocalcin to hydroxyapatite -Pb exposure has been shown to both increase and decrease binding of osteocalcin to hydroxyapatite.
Parvalbumin	Unclear; may buffer Ca^{2+} levels; expressed at high levels in interneurons	No data -Ca^{2+} displacement shown *in vitro*.
Phospholipase A_2	Hydrolyze fatty acids from membrane phospholipids; released fatty acids are metabolized to bioactive lipid mediators	No data -Ca^{2+} displacement shown *in vitro*.

Table 2-49. Effects of Lead (Pb) on Function of Various Proteins

Protein	General function	Effect of Pb; summary of evidence
Protein kinase C (PKC)	Cell signaling, especially growth and differentiation	Increases or decreases activity -Pb shown to activate PKC *in vitro* in bovine adrenal chromaffin cells, rat brain microvessels, human erythrocytes, and rabbit mesenteric arteries. -Pb decreased PKC activity in mouse macrophages and rat brain cortex.
Synaptotagmin I	Ca^{2+} sensor regulating neurotransmitter release	No data -Ca^{2+} displacement shown *in vitro.*
Troponin C	Ca^{2+} sensor regulating muscle contraction	No data -Ca^{2+} displacement shown *in vitro*.
Heme-dependent proteins		
Catalase	Antioxidant; scavenger of hydrogen peroxide	Increases or decreases activity -Pb shown to increase activity in some studies and decrease activity in others, possibly due to differences in species, exposure duration, dose, or other study design variations.
Guanylate cyclase	Catalyzes synthesis of cGMP, which stimulates vasorelaxation in vascular tissues	Impairs production of cGMP -Pb reduced cGMP in plasma and urine of rats exposed by drinking water. -Pb decreased protein levels of soluble guanylate cyclase in vascular tissue.
Hemoglobin	Oxygen transportation	Impairs heme production needed for synthesis of hemoglobin -Pb binding to hemoglobin demonstrated in human blood.
Magnesium-dependent proteins		
Adenine and hypoxanthine/guanine phosphoribosyltransferases	Recycling of nucleotides	Inhibits activity -Pb inhibited phosphoribosyltransferase activities in erythrocytes of rats exposed via drinking water and in human erythrocytes in vitro.
NAD synthetase (Mg)	Nucleotide biosynthesis	Decreases activity -Blood Pb was inversely correlated with NAD synthetase activity in humans.
Pyrimidine 5'-nucleotidase	Dephosphorylates pyrimidine nucleotides in erythrocytes, preserving purine nucleotides (e.g., ATP, ADP) necessary for energy	Alters protein conformation and amino acid positioning at active site, possibly by occupying active site -Pb binding and protein conformation changes observed *in vitro*. -Pyrimidine nucleotide accumulation in erythrocytes is seen in lead poisoning.

Table 2-49. Effects of Lead (Pb) on Function of Various Proteins

Protein	General function	Effect of Pb; summary of evidence
Zinc-dependent proteins		
δ-ALA (δ-ALAD or porphobilinogen synthase)	Heme biosynthesis (converts δ-ALA to porphobilinogen)	Depletes δ-ALAD, preventing heme biosynthesis and leading to accumulation of δ-ALA. - δ-ALAD shown to be major binding target of Pb in erythrocytes.
GATA zinc finger proteins	Activation/suppression of DNA transcription	Decreases ability of GATA proteins to bind to DNA and regulate transcription -Pb binding to cysteine residues and displacement of Zn from GATA proteins observed *in vitro*. -Pb-bound GATA proteins exhibited reduced DNA binding.
Transcription factors TFIIIA, Sp1, and Erg-1	Activation/suppression of DNA transcription	Decreases ability of TFIIIA, Sp1, and Erg-1 to bind to DNA and regulate transcription -Pb exposure caused dissociation of TFIIIA-DNA adducts. -Pb exposure altered DNA binding profile of Sp-1 and Erg-1 in rat pups exposed via lactation, leading to changes in gene expression.
Proteins altered by lead interaction with via other cations or sulfhydryl groups		
ATPases (Ca^{2+}-, Mg^{2+}-, and Na^{+}/K^{+}-)	Ion transport	Decreases activity -Pb decreased ATPase activities in brain, kidneys, liver, testes, and erythrocytes (cells or tissues).
cGMP phosphodiesterase (Zn, Mg)	Hydrolysis of cGMP	Inhibits activity -Decreased activity observed in homogenized bovine retinas exposed to Pb *in vitro*.
Ferrochelatase (Fe)	Heme biosynthesis; incorporates Fe^{2+} into protoporphyrin IX to form heme	Inhibits insertion of Fe into protoporphyrin ring, leading to substitution by Zn -Zn-protoporphyrin levels correlated with blood Pb levels in humans.
Glutathione peroxidase and glutathione S-transferase (Se)	Antioxidants	Reduces uptake of Se and depletes cellular GSH and protein thiols, resulting in altered GST and GPx enzyme activities -Decreased activity, often with compensatory upregulation of the enzymes, seen in Pb-exposed animals and humans.

Table 2-49. Effects of Lead (Pb) on Function of Various Proteins

Protein	General function	Effect of Pb; summary of evidence
Metallothionein (Zn, Cu)	Trace element homeostasis; free radical scavenging	Sequestered by metallothionein, providing protective effect -Pb toxicity is seen at lower blood Pb levels in humans with low expression of metallothionein or low Pb binding to metallothionein. -Pb induced production of metallothionein in mice exposed via intraperitoneal or intravenous injection and in rats exposed via intraperitoneal injection, but not in rats exposed via drinking water. -Presence of zinc metallothionein reduced effect of Pb on membrane integrity in hepatocytes exposed *in vitro*. -Pb nephrotoxicity and preneoplastic and neoplastic lesions in the testes, bladder, and kidneys were more severe or seen at increased incidences in metallothionein-null mice compared with wild-type.
Superoxide dismutase	Antioxidant; catalyzes conversion of superoxide to hydrogen peroxide; inhibits oxidative inactivation of nitric oxide	Increased or decreased activity -Pb shown to increase activity in several studies and decrease activity in others, possibly due to differences in species, exposure duration, dose, or other study design variations.
Thymosin β-4	Actin regulation; exerts angiogenic, anti-inflammatory, and cardioprotective effects on the heart	No data -Pb binding observed *in vitro.*

ADP = adenosine diphosphate; δ-ALA = aminolevulinic acid; δ-ALAD = aminolevulinic acid dehydratase; ATP = adenosine triphosphate; ATPase = family of phosphatase enzymes that breakdown ATP and ADP; cGMP = cyclic guanosine monophosphate; DNA = deoxyribonucleic acid; Erg-1 = early growth response protein 1; GST = glutathione S-transferase; GSH = glutathione; GPx = glutathione peroxidase; NAD = nicotinamide adenine dinucleotide; NAD(P)H = the reduced form of nicotinamide adenine dinucleotide phosphate; ROS = reactive oxygen species; Sp1 = Transcription factor specificity protein 1; TFIIIA = transcription factor IIIA

Sources: EPA 2014c; Ahamed and Siddiqui 2007; Flora et al. 2012; Gonick 2011

otherwise altered by Pb, along with their functions and brief summaries of the evidence for Pb-induced alterations. As the table suggests, Pb-induced alterations in proteins may play a role in its adverse effects on the neurological, hematological, cardiovascular, and skeletal systems.

Through its displacement of calcium, Pb perturbs the function of several calcium-dependent proteins, including protein kinase C, calmodulin, osteocalcin, the mitochondrial transmembrane pore, and NAD(P)H oxidase (reviewed by EPA 2014c). The protein kinase C family of enzymes is important to cell signaling, growth, and differentiation. Pb exposure has been shown to activate PKC in a number of cell types tested *in vitro* (see table), and to decrease its activity in mouse macrophages and rat brain cortex. Pb stimulates calmodulin activity, as shown by increased activity of several calmodulin-dependent enzymes, and increased binding of calmodulin to brain membranes. In experiments testing the affinity of metal cations to bind calmodulin, Pb was more potent than mercury, cadmium, iron, and even calcium. Pb binding to calmodulin has been postulated as a mechanism for it stimulatory effect on Ca^{2+}/Mg^{2+} ATPase. Calmodulin plays an essential role in maintaining calcium homeostasis and regulating calcium-dependent cell signaling important to structural integrity, gene expression, and maintaining membrane potential (reviewed by EPA 2014c).

Skeletal effects of Pb may be mediated in part by Pb's interference with another calcium-dependent protein: osteocalcin (reviewed by EPA 2014c). The binding of Pb to osteocalcin is much stronger than binding of calcium, and Pb binding alters the structure of osteocalcin. The conformational change in osteocalcin induced by Pb has been postulated as the mechanism by which Pb exposure diminishes the adsorption of osteocalcin to hydroxyapatite.

Other calcium-dependent proteins bound to or impaired by Pb include parvalbumin, phospholipase A2, synaptotagmin I (see *Ion Transport* above), troponin C, the mitochondrial transmembrane pore (see Section 2.21.6, Apoptosis), and NAD(P)H oxidase (see Section 2.21.3, Oxidative Stress) (reviewed by EPA 2014c).

Pb also displaces zinc in a number of critical proteins, including ALAD, GATA proteins, and several zinc-binding transcription factors (TFIIIA, Sp1, and Erg-1) (reviewed by EPA 2014c). Section 2.8 provides a detailed discussion of Pb's effects on ALAD and heme biosynthesis. Binding of Pb to zinc-binding domains in GATA proteins and transcription factors inhibits their binding to DNA and impairs their ability to regulate gene expression (see Section 2.21.5, *Epigenetic Effects*, below for further detail).

Through competitive inhibition of magnesium-dependent proteins, Pb also affects the activities of adenine and hypoxanthine/guanine phosphoribosyltransferases, cyclic guanosine monophosphate (cGMP) phosphodiesterase, and pyrimidine 5'-nucleotidase (reviewed by EPA 2014c). In erythrocytes, adenine phosphoribosyltransferase catalyzes the synthesis of nucleotides via the adenine salvage pathway; Pb exposure has been shown to decrease nucleotide pools in human erythrocytes *in vitro* and in erythrocytes from rats exposed via drinking water. Inhibition of cGMP phosphodiesterase, a magnesium-dependent enzyme regulating cGMP signaling in smooth muscle contraction and relaxation, has been observed in homogenized bovine retinas cultured with Pb. Pb inhibits magnesium binding in pyrimidine 5'-nucleotidase, inhibiting its activity by changing its active site conformation. Pyrimidine 5'-nucleotidase occurs at high levels in erythrocytes, where it dephosphorylates pyrimidine nucleotides while leaving purine nucleotides (used as an energy source in erythrocytes, as they lack mitochondria), intact. Basophilic stippling of erythrocytes, a common feature of Pb poisoning, is also seen in individuals with inherited pyrimidine-5'-nucleotidase deficiency (Rees et al. 2003), providing supporting evidence that Pb inactivates the enzyme.

2.21.2 Protein Binding/Sequestration

A number of low molecular-weight proteins, including metallothionein, have been shown to bind (through thiol residues) to Pb, forming inclusion bodies in the kidney, liver, lung, and glial cells (reviewed by EPA 2014c; Gonick 2011). In the case of metallothionein, the effect of the binding is to sequester Pb, protecting the exposed cells and tissues. The strongest evidence for the protective effect of metallothionein comes from studies of metallothionein-null mice, which exhibit more severe Pb-induced renal toxicity, as well as increased incidences of neoplastic and nonneoplastic lesions in the testes, bladder, and kidneys, compared with wild-type mice. Supporting this finding is the observation that higher blood Pb levels, as well as more pronounced Pb-induced effects on systolic blood pressure and kidney function, were observed in exposed workers with a metallothionein mutation (compared with those exhibiting normal metallothionein genotype). Metallothionein levels have been shown to be induced by Pb exposure in mice and in rats pretreated with zinc.

In erythrocytes, the major Pb-binding protein is ALAD; hemoglobin also binds Pb (reviewed by EPA 2014c; Gonick 2011). In exposed humans, polymorphisms in the ALAD gene that increase the Pb-binding capacity of its protein product (e.g., ALAD-2) were observed to decrease blood Pb levels and biomarkers for Pb toxicity, including plasma levulinic acid, zinc protoporphyrin, cortical bone Pb levels,

and dimercaptosuccinic acid-chelatable Pb levels. Other proteins that bind Pb in erythrocytes include pyrimidine 5'-nucleotidase and acyl-coenzyme A binding protein.

In rat kidneys, inclusion bodies consisting of Pb-bound proteins have been observed in a number of studies (reviewed by EPA 2014c; Gonick 2011). These inclusion bodies are initially observed in the cytosol, but appear to translocate to the nucleus, as they disappear concomitantly with the appearance of intranuclear inclusion bodies. The primary Pb-bound protein in the kidney (a 32 kDa protein with an isoelectric point of 6.3, named p32/6.3) has not been identified, but has been shown to be enriched in the brain and is highly conserved across species (rats, mice, dogs, chickens, and humans). Studies in rats exposed by food or drinking water showed that p32/6.3 is not found in the kidneys of untreated rats but rather is induced by Pb exposure. Other Pb-binding proteins identified in the kidneys of rats or humans include acyl-CoA binding protein and thymosin β-4 (the latter is involved in actin regulation).

2.21.3 Oxidative Stress

Pb exposure has resulted in oxidative damage in several tissues in humans and rats, including the brain, kidneys, reproductive organs, heart, and erythrocytes (reviewed by EPA 2014c; Ahamed and Siddiqui 2007). Oxidative damage may play a role in Pb-induced toxicity in these tissues, including neurological effects, hypertension and other cardiovascular effects, and diminished fertility. Pb induces oxidative stress through several mechanisms, including increased production of ROS via inhibition of heme biosynthesis and activation of NAD(P)H oxidase; stimulation of lipid peroxidation and alteration of lipids enhancing their susceptibility to lipid peroxidation; and inactivation and/or depletion of antioxidant enzymes. Through the increased production of ROS, which sequesters nitric oxide, Pb exposure also leads to perturbation of nitric oxide signaling that is critical to vasodilation.

Exposure to Pb triggers increased production of ROS via its effects on heme biosynthesis. In erythrocytes, Pb has been shown to bind to δ-ALAD as well as to inhibit its activity by interfering with the zinc ions the enzyme requires for heme biosynthesis; in fact, inhibition of δ-ALAD activity is inversely correlated with PbB levels in humans (reviewed by EPA 2014c; Ahamed and Siddiqui 2007). δ-ALAD catalyzes the conversion of δ-ALA to porphobilinogen; thus, its inhibition results in accumulation of δ-ALA in blood and in urine. In these environments, δ-ALA undergoes autoxidation, yielding superoxide and hydroxyl radicals, as well as hydrogen peroxide and an ALA radical. In addition, through subsequent reduction of ferricytochrome c and transfer of electrons from oxyhemoglobin, methemoglobin, and ferric and ferrous iron complexes, oxidized δ-ALA also produces ROS.

Pb may also increase intracellular ROS by upregulating expression of NAD(P)H oxidase, an enzyme that produces superoxide anion via reaction of NAD(P)H and molecular oxygen, but data are limited (reviewed by EPA 2014c). Increased protein expression of the glycosylated subunit of NAD(P)H oxidase was observed in tissues of rats exposed to Pb in drinking water, and in human endothelial cells *in vitro*.

ROS produced via Pb effects on δ-ALA and/or NAD(P)H oxidase can damage membrane lipids through peroxidation. In addition, however, Pb has been shown to catalyze ferrous ion-initiated lipid peroxidation (reviewed by EPA 2014c). Furthermore, there is evidence that Pb exerts effects on membrane lipids that render them more vulnerable to peroxidation (reviewed by EPA 2014c; Ahamed and Siddiqui 2007). For example, Pb has been shown to alter the composition of fatty acids in chicks exposed by drinking water, such that a higher fraction of longer fatty acids (such as arachidonic acid) and lower fraction of shorter fatty acids (compared with controls) were observed. Oxidative potential of fatty acids is correlated with both length and desaturation (i.e., the number of double bonds; the hydrogen on a double bond is easier to remove). It has been proposed that Pb may stimulate both elongation and desaturation of fatty acids, increasing their susceptibility to peroxidation. Alterations in lipid composition may also affect membrane permeability and functions, including the activity of membrane-associated enzymes, solute transport functions, endo- and exocytosis, and signal transduction.

Increased circulating ROS (specifically, superoxide anion) can inactivate nitric oxide, an endogenously produced molecule that plays an important role in vasodilation (reviewed by EPA 2014c). Depletion of nitric oxide has been observed in animals exposed to Pb, as well as in human and animal immune cells treated *in vitro*. In addition, nitric oxide depletion is believed to be the mechanism behind Pb-induced upregulation of nitric oxide synthases seen in vascular tissues after Pb exposure. Nitric oxide depletion occurs when it reacts with superoxide anion to form the highly reactive peroxynitrite anion, which itself damages DNA and proteins. Levels of nitrotyrosine, which results from peroxynitrite-induced nitration of tyrosine residues in proteins, were increased in plasma and other tissues after *in vivo* exposure to Pb. In vascular tissues, nitric oxide induces vasorelaxation via cGMP signaling (reviewed by EPA 2014c). Exposure of rats to Pb in drinking water for 1–3 months markedly reduced cGMP levels in both blood and urine. Synthesis of cGMP is catalyzed by soluble guanylate cyclase, a heme-dependent enzyme. Pb exposure has been shown to reduce protein levels of soluble guanylate cyclase in vascular tissues; alleviation of this effect by antioxidant treatment (ascorbic acid) demonstrated that this finding was mediated, at least in part, by increased oxidative stress.

In human epidemiological studies, the ratio of oxidized glutathione (glutathione disulfide or GSSG) to reduced glutathione (GSH), a measure of oxidative stress, was positively correlated with blood Pb levels (reviewed by EPA 2014c; Ahamed and Siddiqui 2007; Flora et al. 2012). The effects of Pb on oxidative stress levels may occur through depletion of antioxidant levels in addition to stimulation of ROS, as oxidative stress occurs when the antioxidant capacity of the body is exceeded. Pb forms covalent bonds with sulfhydryl groups in antioxidant enzymes such as GSH, glutathione reductase (GR), and glutathione S-transferase (GST) (reviewed by EPA 2014c; Ahamed and Siddiqui 2007; Flora et al. 2012). In humans, animals, and *in vitro* studies, decreased GSH in blood and organs has been associated with Pb exposure. After long-term exposure to Pb, increased GSH levels, attributed to compensatory upregulation of GSH biosynthesis, have been reported. Like GSH, GR (which reduces GSSG back to GSH) and GST also have disulfides at their active site that could be bound by Pb. Studies examining GR and GST activity after Pb exposure used varying study designs and showed both increases and decreases; it is not clear whether the differences in results reflect species, strain, dose, or duration differences.

Pb's capacity to compete with cations and its interference with heme biosynthesis have also been suggested as potential mechanisms for its ability to alter levels of SOD, CAT, GPx, and GST (reviewed by EPA 2014c; Flora et al. 2012; Ahamed and Siddiqui 2007). SOD forms require copper, zinc, or manganese, cations that Pb may displace, while catalase is a heme-dependent enzyme. Several studies in humans and animals have shown alterations in SOD and CAT activity, with some evidence for a nonlinear dose-response relationship. EPA (2014c) suggested that increased SOD and CAT may occur at low doses as a result of ROS generation by Pb, while at higher doses, Pb may inactivate the enzymes. Pb exposure also alters activities of GPx and GST, potentially by reducing the uptake of selenium (required by GPx) and/or disrupting protein thiols (necessary for GST function). Decreased GPx and GST activities have been observed, along with compensatory upregulation of these enzymes, in Pb-exposed humans and animals.

2.21.4 Inflammation

Increasing oxidative stress through ROS generation and depletion of antioxidant enzymes may be one mechanism by which Pb induces an inflammatory response (reviewed by EPA 2014c). Inflammation, considered a hallmark of Pb exposure (EPA 2014c), may also be triggered by pro-inflammatory signaling and cytokine production. Inflammation has been seen after Pb exposure in many different cell types, as well as in the kidneys of rats exposed to Pb in drinking water.

Oxidative stress is known to activate the pro-inflammatory nuclear transcription factor kappa B (NFκB) In the rat kidney, Pb-induced inflammation was accompanied by activation of NFκB as well as lymphocyte and macrophage infiltration (reviewed by EPA 2014c). Pb has been shown to stimulate the expression of pro-inflammatory signal mediators including NFκB, activator protein-1 (AP-1), and c-Jun, and to stimulate phosphorylation of the Erk/MAPK pathway. In addition, exposure to Pb is associated with increased production of prostaglandins, which also mediate pro-inflammatory messaging. Increases in arachidonic acid production, leading to increases in prostaglandins E2 and F2 and thromboxane levels, have been seen in Pb-exposed workers as well as in animals and in cultured cells systems exposed to Pb. In vascular smooth muscle cells, Pb has been shown to activate phospholipase A2, which may explain its ability to stimulate the release of arachidonic acid.

In both human epidemiological and laboratory animal studies, Pb exposure has been demonstrated to increase cytokine production (reviewed by EPA 2014c). In these studies, a fairly consistent picture of decreasing Th-1 cytokines and increasing Th-2 cytokines has emerged. EPA (2014c) outlined three modes by which Pb influences cytokine production: (1) direct action on macrophages to increase pro-inflammatory cytokines such as TNF-α and interleukin 6 (IL-6); (2) skew the ratio of IL-12 to IL-10, leading to suppression of Th-1 cell responses and stimulate Th-2 cell responses; and (3) during acquired immune response occurring after Pb exposure, production of cytokines by Th-1 lymphocytes is suppressed, and Th-2 cytokines are increased. The net result of these changes is consistent with the pro-inflammatory picture seen with Pb exposure.

Human epidemiological studies have provided evidence that Pb exposure skews immune responses toward Th-2 pro-inflammatory responses (reviewed by EPA 2014c). Higher blood Pb levels in children were associated with increased serum levels of Il-4 (which induces differentiation of Th0 cells to the Th-2 phenotype) and lower levels of interferon gamma (IFN-γ). In adult students in Korea, higher blood Pb levels were positively associated with increased TNF-α and IL-6; a 1 μg/dL increase in blood Pb was associated with a 23% increase in log TNF-α and a 26% increase in IL-6. Finally, in occupationally-exposed workers, higher blood Pb levels were associated with increases in IL-2, IL-10, IL-6, TNF-α, and granulocyte colony stimulating factor (G-CSF), and, in one study, lower levels of Th1 cytokines IL-1β and IFN-γ. Similar effects were seen in mice exposed to Pb in feed; blood levels of Th-1 cytokines (IL-2 and IFN-γ) were decreased at low dietary doses, while increases in IL-4 were seen as the Pb dose increased. Based on these data, EPA (2014c) suggested that the immune system response to Pb may exhibit nonlinearities at low doses. In rats exposed to Pb via intraperitoneal injection, increased levels of

TNF-α were seen in the hippocampus, and increased IL-6 was noted in the forebrain. *In vitro* data have also shown alterations in cytokine production after exposure to Pb.

2.21.5 Epigenetic Effects

In a small number of studies, Pb has been shown to induce epigenetic effects, including perturbations in DNA methylation as well as alterations in mitogenesis (reviewed by EPA 2014c; Bakulski et al. 2013). In human studies, maternal blood Pb was correlated with decreased DNA methylation of Alu retrotransposable elements in umbilical cord blood, and bone Pb levels were correlated with decreased DNA methylation of LINE-1 retrotransposons in elderly men, while higher blood Pb was associated with increased methylation of p16 tumor suppressor gene promoters in occupationally exposed individuals. Other evidence for effects of Pb on DNA methylation include a study in primates in which the activity of DNA methyltransferase 1 was decreased by early life Pb exposure, and *in vitro* data showing decreased global DNA methylation in rat pheochromocytoma cells. Hypomethylation of DNA has been shown to trigger changes in gene expression that may lead to alterations in tissue differentiation.

Pb exposure also induces effects on mitogenesis, including both increases in cell proliferation and decreases in some systems (reviewed by EPA 2014c). Increased cell proliferation and/or DNA synthesis have been reported in workers exposed to Pb, in hepatocytes of rats exposed by intravenous injection of Pb nitrate, and in mouse lung after exposure to Pb acetate via inhalation. In *in vitro* studies, results were mixed: in some cases cell proliferation was decreased, as Pb exposure resulted in cell cycle arrest. Effects of Pb exposure on gene expression have been demonstrated in several studies (reviewed by EPA 2014c). Although the exact mechanisms by which Pb alters gene expression have not been elucidated, Pb is known to interfere with GATA proteins and several transcription factors (TFIIIA, Sp1, and Erg-1) through its interaction with zinc-binding domains, reducing the ability of these proteins to bind to DNA and exert their transcriptional regulation functions. *In vivo* and *in vitro* studies have shown that Pb alters the transcription of genes for metabolic enzymes including GST-P and GST-Ya, CYPs 1A1 and 1A2, and NAD(P)H:quinone oxidoreductase, as well as genes involved in the pentose phosphate pathway and amino acid metabolism.

2.21.6 Apoptosis

As discussed earlier, Pb is capable of opening the mitochondrial transmembrane pore (MTMP, the first step in the mitochondrial apoptosis cascade), possibly by displacing calcium on the matrix side of the pore (reviewed by EPA 2014c). Evidence for this effect includes observations of mitochondrial swelling

and decreased membrane potential in rat primary cerebellar granule neuronal cultures, astroglia, proximal tubule cells, and retinal rod photoreceptor cells. In addition, release of cytochrome c and activation of caspases 3 and 9 were observed in rat retinal rod cells and hepatic oval cells exposed to Pb *in vitro*. In lymphocytes of Pb-exposed humans, increased apoptosis, karyorrhexis, and karyolysis (early indicators of apoptosis) were observed. Other tissues have also exhibited increased apoptosis after Pb exposure, including liver, fibroblasts, and alveolar macrophages.

CHAPTER 3. TOXICOKINETICS, SUSCEPTIBLE POPULATIONS, BIOMARKERS, CHEMICAL INTERACTIONS

3.1 TOXICOKINETICS

Overview. The toxicokinetics of Pb in humans has been extensively studied and several models have been published that simulate the absorption and complex distribution and elimination of Pb from blood, soft tissues, and bone.

- Absorption:
  - Respiratory tract: Inorganic Pb in submicron size particles can be almost completely absorbed through the respiratory tract, whereas larger particles may be swallowed after deposition in the respiratory tract and upward mucociliary clearance.
  - Gastrointestinal tract: The fraction of ingested Pb absorbed from the gastrointestinal tract depends on many factors, including age, diet, nutrition, and physiological characteristics of Pb in the medium ingested.
  - Children can absorb 40–50% of an oral dose of water-soluble Pb compared to 3–10% for adults.
  - Gastrointestinal absorption of inorganic Pb occurs primarily in the duodenum by saturable mechanisms.
  - Dermal: Inorganic Pb can be absorbed following inhalation, oral, and dermal exposure, but the latter route is much less efficient than the former two. Studies in animals have shown that organic Pb is absorbed through the skin.

- Distribution:
  - The distribution of Pb in the body is route-independent and, in adults, approximately 94% of the total body burden of Pb is in the bones compared to approximately 73% in children.
  - Pb in blood is primarily in red blood cells. Conditions such as pregnancy, lactation, menopause, and osteoporosis increase bone resorption and consequently also increase Pb in blood.
  - Pb can be transferred from the mother to the fetus and also from the mother to infants via maternal milk.

- Metabolism:
  - Metabolism of inorganic Pb consists of formation of complexes with a variety of protein and nonprotein ligands.
  - Organic Pb compounds are actively metabolized in the liver by oxidative dealkylation by P-450 enzymes.

- Excretion:
  - Pb is excreted primarily in urine and feces regardless of the route of exposure. Minor routes of excretion include sweat, saliva, hair, nails, breast milk, and seminal fluid.
  - The elimination half-lives for inorganic Pb in blood and bone are approximately 30 days and 27 years, respectively.

- Toxicokinetics models:
  - Several models of Pb pharmacokinetics have been proposed to characterize such parameters as intercompartmental Pb exchange rates, retention of Pb in various tissues, and relative rates of distribution among the tissue groups.
  - Some models are currently being used or are being considered for broad application in Pb risk assessment.

3.1.1 Absorption

Inhalation Exposure

Inorganic Pb. Inorganic Pb in ambient air consists of aerosols of particulates that can be deposited in the respiratory tract when the aerosols are inhaled. Amounts and patterns of deposition of particulate aerosols in the respiratory tract are affected by the size of the inhaled particles, age-related factors that determine breathing patterns (e.g., nose versus mouth breathing), airway geometry, and air-stream velocity within the respiratory tract (James et al. 1994). Absorption of deposited Pb is influenced by particle size and solubility as well as the pattern of regional deposition within the respiratory tract. Larger particles (>2.5 μm) that are deposited in the ciliated airways (nasopharyngeal and tracheobronchial regions) can be transferred by mucociliary transport into the esophagus and swallowed. Smaller particles (2.5 to <1 μm), which can be deposited in the alveolar region, can be absorbed after extracellular dissolution or ingestion by phagocytic cells (Bailey and Roy 1994).

Deposition in, and clearance from, the respiratory tract have been measured in adult humans (Chamberlain et al. 1978; Hursh and Mercer 1970; Hursh et al. 1969; Morrow et al. 1980; Wells et al. 1975). In these studies, exposures were to Pb-bearing particles having mass median aerodynamic diameters (MMADs) below 1 μm and, therefore, deposition of the inhaled Pb particles can be assumed to have been primarily in the bronchiolar and alveolar regions of the respiratory tract (James et al. 1994) where transport of deposited Pb to the gastrointestinal tract is likely to have been only a minor component of particle clearance (Hursh et al. 1969). Approximately 25% of inhaled Pb chloride or Pb hydroxide (MMAD 0.26 and 0.24 μm, respectively) was deposited in the respiratory tract in adult subjects who inhaled an inorganic Pb aerosol through a standard respiratory mouthpiece for 5 minutes (Morrow et al. 1980). Approximately 95% of deposited inorganic Pb that was inhaled as submicron particles was absorbed (Hursh et al. 1969; Wells et al. 1975). Rates of clearance from the respiratory tract of inorganic Pb inhaled as submicron particles of Pb oxide, or Pb nitrate, were described with half-times ($t_{1/2}$) of 0.8 hours (22%), 2.5 hours (34%), 9 hours (33%), and 44 hours (12%) (Chamberlain et al. 1978). These rates are thought to represent, primarily, absorption from the bronchiolar and alveolar regions of the respiratory tract. Absorption half-times have been estimated in adults who inhaled aerosols of Pb and bismuth isotopes generated from decay of ^{220}Rn or ^{222}Rn (Butterweck et al. 2002; Marsh and Birchall 1999). The absorption half-time was approximately 10 hours in subjects who inhaled aerosols having an activity median particle diameter of approximately 160 nm (range 50–500 nm), and approximately 68 minutes for aerosols having diameters of approximately 0.3–3 nm.

Rates and amounts of absorption of inhaled Pb particles >2.5 μm will be determined, primarily by rates of transport to and absorption from the gastrointestinal tract. Absorption of Pb from the gastrointestinal tract varies with the chemical form ingested, age, meal status (e.g., fed versus fasted), and nutritional factors (see Section 3.1.1 *Oral Exposure*).

Organic Pb. Following a single exposure to vapors of radioactive (^{203}Pb) tetraethyl Pb (approximately 1 mg/m^3 breathed through a mouthpiece for 1–2 minutes) in four male subjects, 37% of inhaled ^{203}Pb was initially deposited in the respiratory tract, of which approximately 20% was exhaled in the subsequent 48 hours (Heard et al. 1979). One hour after the exposure, approximately 50% of the ^{203}Pb burden was associated with liver, 5% was associated with kidney, and the remaining burden was widely distributed throughout the body (determined by external gamma counting), suggesting near complete absorption of the Pb that was not exhaled. In a similar experiment conducted with (^{203}Pb) tetramethyl Pb, 51% of the inhaled ^{203}Pb dose was initially deposited in the respiratory tract, of which approximately 40% was

exhaled in 48 hours. The distribution of ^{203}Pb 1 hour after the exposure was similar to that observed following exposure to tetraethyl Pb.

The relatively rapid and near complete absorption of tetraalkyl Pb that is inhaled and deposited in the respiratory tract is also supported by studies conducted in animal models (Boudene et al. 1977; Morgan and Holmes 1978).

Oral Exposure

Inorganic Pb. The extent and rate of gastrointestinal absorption of ingested inorganic Pb are influenced by physiology (e.g., age, fasting, nutritional calcium and iron status, pregnancy), physicochemical characteristics of the medium ingested (e.g., particle size, mineralogy, solubility, and Pb species) and the ingested Pb dose.

Mechanisms of Absorption. Gastrointestinal absorption of inorganic Pb occurs primarily in the duodenum (Mushak 1991). The exact mechanisms of absorption are unknown and may involve active transport and/or diffusion through intestinal epithelial cells (transcellular) or between cells (paracellular), and may involve ionized Pb (Pb^{+2}) and/or inorganic or organic complexes of Pb. *In vitro* studies of Pb speciation in simulated human intestinal chyme indicate that the concentration of ionized Pb is negligible at Pb concentrations below 10^{-3} M (207 mg/L) and that Pb phosphate and bile acid complexes are the dominant forms when inorganic Pb salts (e.g., Pb nitrate) are added to chyme (Oomen et al. 2003a). However, these complexes may be sufficiently labile to provide ionized Pb for transport across cell membranes (Oomen et al. 2003b). Saturable mechanisms of absorption have been inferred from measurements of net flux kinetics of Pb in *in situ* perfused mouse intestine, *in situ* ligated chicken intestine, and *in vitro* isolated segments of rat intestine (Aungst and Fung 1981; Barton 1984; Flanagan et al. 1979; Mykkänen and Wasserman 1981). By analogy to other divalent cations, saturable transport mechanisms for Pb^{+2} may exist within the mucosal and serosal membranes and within the intestinal epithelial cell. For calcium and iron, these are thought to represent membrane carriers (e.g., Ca^{2+}-Mg^{2+}-ATPase, Ca^{2+}/Na^{+} exchange, DMT1) or facilitated diffusion pathways (e.g., Ca^{2+} channel) and intracellular binding proteins for Ca^{2+} (Bronner et al. 1986; Fleming et al. 1998b; Gross and Kumar 1990; Teichmann and Stremmel 1990).

Effect of Age. Gastrointestinal absorption of water-soluble Pb appears to be higher in children than in adults. Estimates derived from dietary balance studies conducted in infants and children (ages 2 weeks to

8 years) indicate that approximately 40–50% of ingested Pb is absorbed (Alexander et al. 1974; Ziegler et al. 1978). In adults, estimates of absorption of ingested water-soluble Pb compounds (e.g., Pb chloride, Pb nitrate, Pb acetate) ranged from 3 to 10% in fed subjects (Heard and Chamberlain 1982; James et al. 1985; Rabinowitz et al. 1980; Watson et al. 1986). Data available on Pb absorption between childhood and adulthood ages are very limited. While no absorption studies have been conducted on subjects in this age range, the kinetics of the change in stable isotope signatures of blood Pb in mothers and their children, as both come into equilibrium with a novel environmental Pb isotope profile, suggest that children ages 6–11 years and their mothers may absorb a similar percentage of ingested Pb (Gulson et al. 1997b).

Studies in experimental animals provide additional evidence for an age-dependency of gastrointestinal absorption of Pb. Absorption of Pb, administered as Pb acetate (6.37 mg Pb/kg, gavage), was higher in juvenile Rhesus monkeys (38% of dose) compared to adult female monkeys (26% of the dose) (Pounds et al. 1978). Rat pups absorb approximately 40–50 times more Pb from the diet than do adult rats (Aungst et al. 1981; Forbes and Reina 1972; Kostial et al. 1978). This age difference in absorption may be due, in part, to the shift from the neonatal to adult diet, and to postnatal physiological development (enzymes, transporters, gastric pH) of the gastrointestinal tract (Weis and LaVelle 1991).

Effect of Fasting. The presence of food in the gastrointestinal tract decreases absorption of water-soluble Pb (Blake and Mann 1983; Blake et al. 1983; Heard and Chamberlain 1982; James et al. 1985; Maddaloni et al. 1998; Rabinowitz et al. 1980). In adults, absorption of a tracer dose of Pb acetate in water was approximately 63% when ingested by fasted subjects and 3% when ingested with a meal (James et al. 1985). Heard and Chamberlain (1982) reported nearly identical results. The arithmetic mean of reported estimates of absorption in fasted adults was 57% (calculated by ATSDR based on Blake et al. 1983; Heard and Chamberlain 1982; James et al. 1985; Rabinowitz et al. 1980). Reported fed/fasted ratios for absorption in adults range from 0.04 to 0.2 (Blake et al. 1983; Heard and Chamberlain 1983; James et al. 1985; Rabinowitz et al. 1980). Mineral content is one contributing factor to the lower absorption of Pb when Pb is ingested with a meal; in particular, the presence of calcium and phosphate in a meal will depress the absorption of ingested Pb (Blake and Mann 1983; Blake et al. 1983; Heard and Chamberlain 1982). Suppression of absorption by meals may explain the observation of lower PbB in children (age 3–5 years) who ate breakfast compared to children who went without breakfast, after controlling for nutritional variables (Liu et al. 2011).

Effect of Nutrition. Pb absorption in children is affected by nutritional iron status. Children who are iron deficient have higher PbBs than similarly exposed children who are iron replete, which would suggest that iron deficiency may result in higher absorption of Pb or, possibly, other changes in Pb biokinetics that would contribute to lower PbBs (Mahaffey and Annest 1986; Marcus and Schwartz 1987). Genetic variation in genes involved in iron metabolism appear to affect PbBs; however, it is not certain if these associations are caused by changes in Pb absorption. These include variants in the hemochromatosis (HFE) and transferrin genes, which have been associated with higher PbBs in children (Hopkins et al. 2008), and with lower PbBs and bone Pb levels in elderly men (Wright et al. 2004).

Evidence for the effect for iron deficiency on Pb absorption has been provided from animal studies. In rats, iron deficiency increases the gastrointestinal absorption of Pb, possibly by enhancing binding of Pb to iron binding proteins in the intestine (Bannon et al. 2003; Barton et al. 1978b; Morrison and Quaterman 1987). Interactions between iron and Pb appear to involve either intracellular transfer or basolateral transfer mechanisms. Iron ($FeCl_2$) added to the mucosal fluid of the everted rat duodenal sac decreases serosal transfer, but not mucosal uptake of Pb (Barton 1984). When mRNA for DMT1, a mucosal membrane carrier for iron (which also transports other divalent metal cations), was suppressed in Caco 2 cells (a human gastrointestinal cell line), the rate of iron and cadmium uptake decreased by 50% compared to cells in which DMT1 mRNA was not suppressed; however, DMT1 mRNA suppression did not alter the rate of Pb uptake by Caco 2 cells, indicating that Pb may enter Caco 2 cells through a mechanism that is independent of DMT1 (Bannon et al. 2003). The above observations suggest that rate-limiting saturable mechanisms for Pb absorption are associated with transfer of Pb from cell to blood rather than with mucosal transfer. Similar mechanisms may contribute to Pb-iron and Pb-calcium absorption interactions in humans, and possibly interactions between Pb and other divalent cations such as cadmium, copper, magnesium, and zinc.

Dietary calcium intake affects Pb absorption. An inverse relationship has been observed between dietary calcium intake and PbBs in children, suggesting that children who are calcium-deficient may absorb more Pb than calcium-replete children (Elias et al. 2007; Mahaffey et al. 1986; Schell et al. 2004; Ziegler et al. 1978). An effect of calcium on Pb absorption is also evident in adults. In experimental studies of adults, absorption of a single dose of Pb (100–300 µg Pb chloride) was lower when the Pb was ingested together with calcium carbonate (0.2–1 g calcium carbonate) than when the Pb was ingested without additional calcium (Blake and Mann 1983; Heard and Chamberlain 1982). A similar effect of calcium occurs in rats (Barton et al. 1978a). Complexation with calcium (and phosphate) in the gastrointestinal tract and competition for a common transport protein have been proposed as possible mechanisms for this

interaction (Barton et al. 1978a; Heard and Chamberlain 1982). Absorption of Pb from the gastrointestinal tract is enhanced by dietary calcium depletion or administration of cholecalciferol (Mykkänen and Wasserman 1981, 1982). This "cholecalciferol-dependent" component of Pb absorption appears to involve a stimulation of the serosal transfer of Pb from the epithelium, not stimulation of mucosal uptake of Pb (Mykkänen and Wasserman 1981, 1982). This is similar to the effects of cholecalciferol on calcium absorption (Bronner et al. 1986; Fullmer and Rosen 1990).

In a study of young children (ages 6–12 months), PbBs increased in association with lower dietary Zn levels (Schell et al. 2004); however, it is not certain if these associations were caused by changes in Pb absorption.

Effect of Pregnancy. Absorption of Pb may increase during pregnancy. Although there is no direct evidence for this in humans, an increase in Pb absorption may contribute, along with other mechanisms (e.g., increased mobilization of bone Pb), to the increase in PbBs that has been observed during the latter half of pregnancy (see Section 3.1.2, *Pb Distribution during Pregnancy and Maternal-Fetal-Infant Transfer*).

Effect of Dose. Pb absorption in humans may be a capacity-limited process, in which case, the percentage of ingested Pb that is absorbed may decrease with increasing rate of Pb intake. Studies, to date, do not provide a firm basis for discerning if the gastrointestinal absorption of Pb is limited by dose. Numerous observations of nonlinear relationships between PbB and Pb intake in humans provide support for the existence of a saturable absorption mechanism or some other capacity-limited process in the distribution of Pb in humans (Pocock et al. 1983; Sherlock and Quinn 1986; Sherlock et al. 1984) (see Section 3.1.2, *Pb in Blood* and *Pb in Plasma* for discussion of saturable uptake of Pb in red blood cells). However, in immature swine that received oral doses of Pb in soil, Pb dose-blood Pb relationships were curvilinear, whereas dose-tissue Pb relationships for bone, kidney, and liver were linear. The same pattern (nonlinearity for PbB and linearity for tissues) was observed in swine administered Pb acetate intravenously (Casteel et al. 1997, 2006). These results suggest that the nonlinearity in the Pb dose-blood Pb relationship may derive from an effect of Pb dose on some aspect of the biokinetics of Pb other than absorption. In fasted rats, absorption was estimated at 42 and 2% following single oral administration of 1 and 100 mg Pb/kg, respectively, as Pb acetate, suggesting a limitation on absorption imposed by dose (Aungst et al. 1981). Evidence for capacity-limited processes at the level of the intestinal epithelium (Aungst and Fung 1981; Barton 1984; Flanagan et al. 1979; Mykkänen and Wasserman 1981) suggests

that the intake-uptake relationship for Pb is likely to be nonlinear; however, the dose at which absorption becomes appreciably limited in humans is not known.

Effect of Particle Size. Particle size influences the degree of gastrointestinal absorption (Ruby et al. 1999). In rats, an inverse relationship was found between absorption and particle size of Pb in diets containing metallic Pb particles that were ≤250 μm in diameter (Barltrop and Meek 1979). Tissue Pb concentration was a 2.3-fold higher when rats ingested an acute dose (37.5 mg Pb/kg) of Pb particles that were <38 μm in diameter than when rats ingested particles having diameters in the range of 150–250 μm (Barltrop and Meek 1979). Dissolution kinetics experiments with Pb-bearing mine waste soil suggest that surface area effects control dissolution rates for particles sizes of <90 μm diameter; however, dissolution of 90–250 μm particle size fractions appeared to be controlled more by surface morphology (Davis et al. 1994). Similarly, Healy et al. (1982) found that the solubility of Pb sulfide in gastric acid *in vitro* was much greater for particles that were 30 μm in diameter than for particles that were 100 μm in diameter.

Absorption from Soil. Absorption of Pb from the gastrointestinal tract involves absorptive transport of soluble Pb species (e.g., Pb^{2+}) across the gastrointestinal tract epithelium. In order for Pb to be absorbed from soil, it must first be made bioaccessible in the gastrointestinal tract. The process of rendering soil Pb bioaccessible may involve: (1) physical and/or chemical digestion of the soil particles to expose Pb deposits to gastrointestinal tract fluids; (2) transfer of Pb minerals from exposed surfaces on soil particles to the aqueous environment of the gastrointestinal tract; and (3) chemical transformation of Pb minerals to soluble Pb species (e.g., Pb^{2+}) that are substrates for absorptive transport. Although absorptive transport of Pb occurs predominantly, if not solely, in the upper small intestine, bioaccessibility processes occurring in the stomach appear to be major determinants of Pb absorption.

Adult subjects who ingested soil (particle size <250 μm) collected from the Bunker Hill National Priorities List (NPL) site absorbed 26% of the resulting 250 μg/70 kg body weight Pb dose when the soil was ingested in the fasted state, and 2.5% when the same soil Pb dose was ingested with a meal (Maddaloni et al. 1998). The value reported for fasted subjects (26%) was approximately half that reported for soluble Pb ingested by fasting adults, or approximately 60% (Blake et al. 1983; Heard and Chamberlain 1983; James et al. 1985; Rabinowitz et al. 1980). Measurements of the absorption of soil Pb in infants or children have not been reported.

Absorption of Pb from ingested soils and surface dust has been studied more extensively in animals (Bannon et al. 2009; Barltrop and Meek 1979; Bradham et al. 2016; Brown et al. 2004; Casteel et al.

1997, 2006; Freeman et al. 1992, 1994, 1996; Healy et al. 1982; Hettiearachchi et al. 2003; Juhasz et al. 2009; Ryan et al. 2004; Weis and Lavelle 1991). These studies have shown that absorption of soil Pb varies depending upon the Pb mineralogy and physical characteristics of the Pb in the soil (e.g., encapsulated or exposed, particle size). Studies conducted in swine and other animal models have provided estimates of relative bioavailability (RBA) of Pb in soils collected from sites impacted by a variety of sources of Pb contamination including ore and ore processing, shooting of Pb munitions, and Pb-based paint (Bannon et al. 2009; Barltrop and Meek 1979; Bradham et al. 2016; Brown et al. 2004; Casteel et al. 1997, 2006; Freeman et al. 1992, 1994, 1996; Healy et al. 1982; Hettiearachchi et al. 2003; Juhasz et al. 2009; Ryan et al. 2004; Weis and Lavelle 1991). RBA is the ratio of the absolute bioavailability (or absorption fraction) of Pb in soil to that of a water-soluble reference (Pb acetate). RBA has been measured in animals models using various approaches, including measurement of blood and tissue Pb in animals following dosing with soil or Pb acetate. RBA estimates from these studies ranged from 1 to 100% (mean 60%, n=33, calculated by ATSDR). RBA for soils from firing ranges where the predominant form of Pb was Pb carbonate were approximately 100% (Bannon et al. 2009). A soil amended with NIST paint standard (a mixture of Pb carbonate and Pb oxide) had an RBA of 92%. Smelter slag and soils in which the dominant source of Pb was smelter slag had relatively low RBA (14 – 40%). Galena (lead sulfide) in soil also had relatively low RBA (1–6%).

Casteel et al. (2006) estimated Pb RBA of 19 soils in swine and categorized the RBA according to Pb mineral associations. Electron microprobe analyses of Pb-bearing grains in the various soils revealed that the grains ranged from as small as 1–2 μm up to a maximum of 250 μm (the sieve size used in preparation of the samples) and that Pb was present in a wide range of different mineral associations (phases), including various oxides, sulfides, sulfates, and phosphates. These variations in size and mineral content of the Pb-bearing grains are the suspected cause of variations in the gastrointestinal absorption of Pb from different samples of soil. Based on these very limited data, the RBA of Pb mineral phases were rank-ordered (Table 3-1).

Table 3-1. Ranking of Relative Bioavailability of Lead (Pb) Mineral Phases in Soil[a]

Low bioavailability (RBA<0.25)	Medium bioavailability (RBA=0.25–0.75)	High bioavailability (RBA>0.75)
Angelsite	Pb oxide	Cerussite
Fe(M) oxide	Pb phosphate	Mn(M) oxide
Fe(M) sulfate		
Galena		
Pb(M) oxide		

[a]Estimates are based on studies of immature swine.

Fe = iron; M = metal; Mn = manganese; RBA = relative bioavailability (compared to Pb acetate)

Source: Casteel et al. 2006

Several studies have shown that elevating the phosphate concentration of soil can decrease soil Pb RBA (Brown et al. 2004; Hettiarachichi et al. 2003; Ryan et al. 2004). The mechanism for the effect is thought to be the formation of a relatively insoluble form of Pb in soil, pyromorphite, which has a low RBA (Scheckel et al. 2013).

Bioaccessibility in Soil and its Relationship to Relative Bioavailability. Empirical evidence supporting the importance of gastric bioaccessibility in Pb absorption comes from studies of relationships between extractability of Pb from soil measured *in vitro* and Pb RBA measured in animals. *In vitro* extractability of Pb from soil (*in vitro* bioaccessibility, IVBA) strongly correlates with RBA measured swine assays when the extraction is performed at gastric pH (r^2=0.92, n=18; Drexler and Brattin 2007). Bioaccessibility estimates obtained from IVBA assays are sensitive to assay conditions such as pH, liquid:soil ratios, inclusion or absence of food material, and differences in methods used to separate dissolved and particle-bound Pb (e.g., centrifugation versus filtration); as a result, different assays can yield different results when applied to the same soils or surface dusts (Juhasz et al. 2011; Lu et al. 2011; Roussel et al. 2010; Saikat et al. 2007; Smith et al. 2011; Van de Wiele et al. 2007). For this reason, application of IVBA assays for predicting RBA must be supported by demonstration of a strong correlation between IVBA and RBA (Drexler and Brattin 2007). Even in the absence of validation of RBA predictions, IVBA assays may be useful for predicting relative differences in RBA between soils. For example, the relative change in Pb RBA resulting from treatment of soils with phosphate amendments was predicted from IVBA measurements even though the IVBA assay performed poorly at predicting the actual RBA of the soils (Juhasz et al. 2016). Bioaccessibility measured with IVBA assays has been shown to increase with decreasing particle size (varied from <2,000 to <50 μm) (Juhasz et al. 2011) and increase with increasing soil acidity and organic matter content (Jin et al. 2005).

Dermal Exposure

Inorganic Pb. Dermal absorption of inorganic Pb compounds is generally considered to be much less than absorption by inhalation or oral routes of exposure; however, few studies have provided quantitative estimates of dermal absorption of inorganic Pb in humans, and the quantitative significance of the dermal absorption pathway as a contributor to Pb body burden in humans remains an uncertainty. Pb was detected in the upper layers of the stratum corneum of Pb-battery workers, prior to their shifts and after cleaning of the skin surface (Sun et al. 2002), suggesting adherence and/or possible dermal penetration of Pb. Following skin application of ^{203}Pb-labeled Pb acetate in cosmetic preparations (0.12 mg Pb in 0.1 mL or 0.18 mg Pb in 0.1 g of a cream) to eight male volunteers for 12 hours, absorption was ≤0.3%, based on whole-body, urine, and blood ^{203}Pb measurements, and was predicted to be 0.06% during normal use of such preparations (Moore et al. 1980). Most of the absorption took place within 12 hours of exposure. Pb also appears to be absorbed across human skin when applied to the skin as Pb nitrate; however, quantitative estimates of absorption have not been reported. Pb (4.4 mg, as Pb nitrate) was applied (vehicle or solvent not reported) to an occluded filter placed on the forearm of an adult subject for 24 hours, after which, the patch was removed, the site cover and the forearm were rinsed with water, and total Pb was quantified in the cover material and rinse (Stauber et al. 1994). The amount of Pb recovered from the cover material and rinse was 3.1 mg (70% of the applied dose). Based on this recovery measurement, 1.3 mg (30%) of the applied dose remained either in the skin or had been absorbed in 24 hours; the amount that remained in or on the skin and the fate of this Pb (e.g., exfoliation) was not determined. Exfoliation has been implicated as an important pathway of elimination of other metals from skin (e.g., inorganic mercury; Hursh et al. 1989). Pb concentrations in sweat collected from the right arm increased 4-fold following the application of Pb to the left arm, indicating that some Pb had been absorbed (amounts of sweat collected or total Pb recovered in sweat were not reported; Stauber et al. 1994). In similar experiments with three subjects, measurements of ^{203}Pb in blood, sweat, and urine, made over a 24-hour period following dermal exposures to 5 mg Pb as ^{203}Pb nitrate or acetate, accounted for <1% of the applied (or adsorbed) dose (Stauber et al. 1994). This study also reported that absorption of Pb could not be detected from measurements of Pb in sweat following dermal exposure to Pb as Pb carbonate.

Information on relative dermal permeability of inorganic and organic Pb salts of Pb comes from studies of *in vitro* preparations of excised skin; the rank ordering of penetration rates through excised human skin

was: Pb nuolate (Pb linoleic and oleic acid complex) > Pb naphthanate > Pb acetate > Pb oxide (nondetectable) (Bress and Bidanset 1991).

Studies conducted in animals provide additional evidence that dermal absorption of inorganic Pb is substantially lower than absorption from the inhalation or oral route. In a comparative study of dermal absorption of inorganic and organic salts of Pb conducted in rats, approximately 100 mg of Pb was applied in an occluded patch to the shaved backs of rats. Based on urinary Pb measurements made prior to and for 12 days following exposure, Pb compounds could be ranked according to the relative amounts absorbed (i.e., percent of dose recovered in urine; calculated by ATSDR): Pb naphthalene (0.17%), Pb nitrate (0.03%), Pb stearate (0.006%), Pb sulfate (0.006%), Pb oxide (0.005%), and metal Pb powder (0.002%). This rank order (i.e., Pb naphthalene > Pb oxide) is consistent with a rank ordering of penetration rates of inorganic and organic Pb salts through excised skin from humans and guinea pigs: Pb nuolate (Pb linoleic and oleic acid complex) > Pb naphthanate > Pb acetate > Pb oxide (nondetectable) (Bress and Bidanset 1991). The estimates for percent of dose excreted underestimate actual absorption as these estimates do not account for the Pb retained in bone and other tissues.

Following application of Pb acetate to the shaved clipped skin of rats, the concentration of Pb in the kidneys was found to be higher relative to controls, suggesting that absorption of Pb had occurred (Laug and Kunze 1948). This study also observed that dermal absorption of Pb from Pb arsenate was significantly less than from Pb acetate, and that mechanical injury to the skin significantly increased the dermal penetration of Pb.

Organic Pb. Relative to inorganic Pb and organic Pb salts, tetraalkyl Pb compounds have been shown to be rapidly and extensively absorbed through the skin of rabbits and rats (Kehoe and Thamann 1931; Laug and Kunze 1948). A 0.75-mL amount of tetraethyl Pb, which was allowed to spread uniformly over an area of 25 cm^2 on the abdominal skin of rabbits, resulted in 10.6 mg of Pb in the carcass at 0.5 hours and 4.41 mg at 6 hours (Kehoe and Thamann 1931). Tetraethyl Pb was reported to be absorbed by the skin of rats to a much greater extent than Pb acetate, Pb oleate, and Pb arsenate (Laug and Kunze 1948). Evidence for higher dermal permeability of organic Pb compounds compared to inorganic organic salts of Pb also comes from *in vitro* studies conducted with excised skin. The rank order of absorption rates through excised skin from humans and guinea pigs was as follows: tetrabutyl Pb > Pb nuolate (Pb linoleic and oleic acid complex) > Pb naphthanate > Pb acetate > Pb oxide (nondetectable) (Bress and Bidanset 1991).

3.1.2 Distribution

Inorganic Pb. Absorbed inorganic Pb appears to be distributed in essentially the same manner regardless of the route of absorption (Chamberlain et al. 1978; Kehoe 1987); therefore, the distribution of absorbed Pb (i.e., by any route) is discussed in this section, rather than in separate sections devoted to specific routes of exposure. The expression "body burden" is used here to refer to the total amount of Pb in the body. Most of the available information about the distribution of Pb to major organ systems (e.g., bone, soft tissues) derives from autopsy studies conducted in the 1960s and 1970s and reflect body burdens accrued during periods when ambient and occupational exposure levels were much higher than current levels (Barry 1975, 1981; Gross et al. 1975; Schroeder and Tipton 1968). A more recent autopsy study found lower Pb concentrations in autopsies performed during the period 2004–2013 (Mari et al. 2014). In general, these studies indicate that the distribution of Pb appears to be similar in children and adults, although a larger fraction of the Pb body burden of adults resides in bone. Several models of Pb pharmacokinetics have been proposed to characterize such parameters as intercompartmental Pb exchange rates, retention of Pb in various tissues, and relative rates of distribution among the tissue groups (see Section 3.1.5 for further discussion of models).

Pb in Blood. Concentrations of Pb in blood vary considerably with age, physiology/life stage (e.g., pregnancy, lactation, menopause), and numerous factors that affect exposure to Pb. PbBs in various demographic strata of the U.S. population are periodically estimated from the NHANES. Based on data from NHANES (2013–2014, CDC 2018a), the geometric mean PbB of U.S. adults, age ≥20 years, was 0.967 μg/dL (95% CI 0.921, 1.02). The geometric mean PbB of U.S. children, age 1–5 years, was 0.782 (95% CI 0.705, 0.868). PbBs in the United States have decreased considerably in the last several decades as a result of removal of Pb from gasoline and restrictions placed on the use of Pb in residential paints (Brody et al. 1994; CDC 2011, 2018a; Pirkle et al. 1994, 1998; Schwartz and Pitcher 1989).

Pb in Red Blood Cells. Pb in blood is primarily in the red blood cells (99%) (Bergdahl et al. 1997a, 1998, 1999; Hernandez-Avila et al. 1998; Manton et al. 2001; Schutz et al. 1996; Smith et al. 2002). Although the mechanisms by which Pb crosses cell membranes have not been fully elucidated, results of studies in intact red blood cells and red blood cell ghosts indicate that there are two, and possibly three, pathways for facilitated transfer of Pb across the red cell membrane. The major proposed pathway is an anion exchanger that is dependent upon HCO_3^- and is blocked by anion exchange inhibitors (Bannon et al. 2000, Simons 1985, 1986a, 1986b, 1993). A second minor pathway, which does not exhibit HCO_3^- dependence and is not sensitive to anion exchange inhibitors, may also exist (Simons 1986b). Pb and

calcium may also share a permeability pathway, which may be a Ca^{2+}-channel (Calderon-Salinas et al. 1999). Pb is transferred out of the erythrocyte by an active transport pathway, most likely a (Ca^{2+}, Mg^{2+})-ATPase (Simons 1988).

Pb in erythrocytes binds to several intracellular proteins. ALAD is the primary binding ligand for Pb in erythrocytes (Bergdahl et al. 1997a, 1998; Sakai et al. 1982; Xie et al. 1998). Pb binding to ALAD is saturable; the binding capacity has been estimated to be approximately 85 µg/dL red blood cells (or approximately 40 µg/dL whole blood) and the apparent dissociation constant has been estimated to be approximately 1.5 µg/L (Bergdahl et al. 1998). Two other Pb-binding proteins have been identified in erythrocytes, a 45 kDa protein (Kd 5.5 µg/L) and a smaller protein(s) having a molecular weight <10 kDa (Bergdahl et al. 1996, 1997a, 1998). Of the three principal Pb-binding proteins identified in erythrocytes, ALAD has the strongest affinity for Pb (Bergdahl et al. 1998) and appears to dominate the ligand distribution of Pb (35–84% of total erythrocyte Pb) at blood Pb levels below 40 µg/dL (Bergdahl et al. 1996, 1998; Sakai et al. 1982). The decrease in hematocrit that occurs in early infancy (51% at birth to 35% at 6 months) may decrease the total binding capacity of blood and PbBs over the first postnatal 6 months (Simon et al. 2007).

Pb binds to and inhibits the activity of ALAD (Gercken and Barnes 1991; Gibbs et al. 1985; Jaffe et al. 2000; Sakai et al. 1982, 1983). Binding of zinc is essential for ALAD activity, and Pb inhibits activity of ALAD by displacing zinc (Jaffe et al. 2000). Synthesis of ALAD appears to be induced in response to inhibition of ALAD and, therefore, in response to binding of Pb to ALAD (Boudene et al. 1984; Fujita et al. 1982). Several mechanisms may participate in the induction of ALAD, including (1) inhibition of ALAD directly by Pb; (2) inhibition by protoporphyrin, secondary to accumulation of protoporphyrin as a result of Pb inhibition of ferrochelatase; and (3) accumulation of ALA (a substrate of ASAD), secondary to inhibition of ALAD, which may stimulate ALAD synthesis in bone marrow cells (Boudene et al. 1984; Fujita et al. 1982).

ALAD is a polymorphic enzyme with two alleles (ALAD 1 and ALAD 2) and three genotypes (ALAD 1,1, ALAD 1,2, and ALAD 2,2) (Battistuzzi et al. 1981, Scinicariello et al. 2007). Numerous studies have examined the relationship between ALAD genotype and PbBs and the results of these studies are mixed with some studies finding higher PbBs in association with the ALAD 2 allele and other studies finding no associations or lower PbBs associated with the ALAD 2 allele (see Section 3.2). One possible mechanism by which ALAD polymorphism could affect PbBs is by allelic variation in Pb binding to

ALAD (Bergdahl et al. 1997b). However, competitive displacement studies with recombinant human ALAD 1 and ALAD 2 did not indicate differences in affinity for Pb relative to zinc (Jaffe et al. 2000).

Pb in Blood Plasma. Pb binds to several constituents in plasma and it has been proposed that Pb in plasma exists in four states: loosely bound to serum albumin or other proteins with relatively low affinity for Pb, complexed to low molecular weight ligands such as amino acids and carboxylic acids, tightly bound to a circulating metalloprotein, and as free Pb^{2+} (Al-Modhefer et al. 1991). Free ionized Pb (i.e., Pb^{2+}) in plasma represents an extremely small percentage of total plasma Pb. The concentration of Pb^{2+} in fresh serum, as measured by an ion-selective Pb electrode, was reported to be 1/5,000 of the total serum Pb (Al-Modhefer et al. 1991). Approximately 40–75% of Pb in the plasma is bound to plasma proteins, of which albumin appears to be the dominant ligand (Al-Modhefer et al. 1991; Ong and Lee 1980). Pb also binds to transferrins and γ-globulins (Guo et al. 2014; Ong and Lee 1980). Pb in serum that is not bound to protein exists largely as complexes with low molecular weight sulfhydryl compounds (e.g., cysteine, homocysteine). Other potential low molecular weight Pb-binding ligands in serum may include citrate, cysteamine, ergothioneine, glutathione, histidine, and oxylate (Al-Modhefer et al. 1991).

Saturable binding to red blood cell proteins contributes to curvature to the blood Pb-plasma Pb relationship with an increase in the plasma/blood Pb ratio with increasing PbB (Barbosa et al. 2006a; Bergdahl et al. 1997b, 1998, 1999; DeSilva 1981; Jin et al. 2008; Kang et al. 2009; Manton et al. 2001; Rentschler et al. 2012; Smith et al. 2002; Tian et al. 2013). The curvature becomes evident at PbBs well above 10 µg/dL. As binding sites for Pb in red blood cells become saturated, a larger fraction of the blood Pb is available in plasma to distribute to brain and other Pb-responsive tissues. This contributes to a curvature in the relationship between Pb intake and PbB, with the blood Pb/intake slope decreasing with increasing Pb intake, which has been observed in children (Sherlock and Quinn 1986) and immature swine (Casteel et al. 2006). Saturable binding of Pb to red blood cell proteins also contributes to a curvilinear relationship between blood Pb and urinary Pb, whereas the relationship between plasma Pb concentration and urine Pb is linear (Bergdahl et al. 1997b).

Pb in Bone. In human adults, approximately >90% of the total body burden of Pb is found in the bones. Based on analyses of post-mortem tissues, bone accounted for 94% of the total Pb body burden of adults and 73% of the body burden in children (Barry 1975). Pb concentrations in bone increase with age, indicative of a relatively slow turnover of Pb in adult bone (Barry 1975, 1981; Gross et al. 1975; Schroeder and Tipton 1968; Wilker et al. 2011). A portion of Pb in bone readily exchanges with the plasma Pb pool and, as a result, bone Pb is a reservoir for replenishment of Pb eliminated from blood by

excretion (Alessio 1988; Behinaein et al. 2012, 2014; Chettle et al. 1991; Hryhorczuk et al. 1985; Nie et al. 2005; Nilsson et al. 1991; Rabinowitz et al. 1976). Pb in adult bone can serve to maintain blood Pb levels long after exposure has ended (Fleming et al. 1997; Inskip et al. 1996; Kehoe 1987; O'Flaherty et al. 1982; Smith et al. 1996). It can also serve as a source of Pb transfer to the fetus when maternal bone is resorbed for the production of the fetal skeleton (Franklin et al. 1997; Gulson et al. 1997b, 1999b, 2003).

Pb forms highly stable complexes with phosphate and can replace calcium in the calcium-phosphate salt, hydroxyapatite, which comprises the primary crystalline matrix of bone (Bres et al. 1986; Lloyd et al. 1975; Meirer et al. 2011; Miyake 1986; Verbeek et al. 1981). As a result, Pb deposits in bone during the normal mineralization process that occurs during bone growth and remodeling and is released to the blood during the process of bone resorption (Aufderheide and Wittmers 1992; O'Flaherty 1991b, 1993). During infancy and childhood, bone calcification is most active in trabecular bone, whereas in adulthood, calcification occurs at sites of remodeling in cortical and trabecular bone. This suggests that Pb accumulation will occur predominantly in trabecular bone during childhood, and in both cortical and trabecular bone in adulthood (Aufderheide and Wittmers 1992). The association of Pb uptake and release from bone with the normal physiological processes of bone formation and resorption renders Pb biokinetics sensitive to these processes. Physiological states (e.g., pregnancy, menopause, advanced age) or disease-related states (e.g., osteoporosis, prolonged immobilization) that are associated with increased bone resorption will tend to promote the release of Pb from bone, which, in turn, may contribute to an increase in the concentration of Pb in blood (Berkowtiz et al. 2004; Bonithon-Kopp et al. 1985; Garrido Latorre et al. 2003; Hernandez-Avila et al. 2000; Jackson et al. 2010; Markowitz and Weinberger 1990; Mendola et al. 2013; Nash et al. 2004; Nie et al. 2009; Popovic et al. 2005; Silbergeld et al. 1988; Symanski and Hertz-Picciotto 1995; Thompson et al. 1985).

Two physiological compartments appear to exist for Pb in cortical and trabecular bone, to varying degrees. In one compartment, bone Pb is essentially inert, having a half-life of several decades. A labile compartment exists as well that allows for maintenance of an equilibrium of Pb between bone and soft tissue or blood (Rabinowitz et al. 1976). Although a high bone formation rate in early childhood results in the rapid uptake of circulating Pb into mineralizing bone, bone Pb is also recycled to other tissue compartments or excreted in accordance with a high bone resorption rate (O'Flaherty 1995a). Thus, most of the Pb acquired early in life is not permanently fixed in the bone (O'Flaherty 1995a). In general, bone turnover rates decrease as a function of age, resulting in slowly increasing bone Pb levels among adults (Barry 1975; Gross et al. 1975; Schroeder and Tipton 1968). Bone Pb burdens in adults are slowly lost by diffusion (heteroionic exchange) as well as by resorption (O'Flaherty 1995a, 1995b). An XRF study of

tibia Pb concentrations in individuals >10 years old showed a gradual increase in bone Pb after age 20 (Kosnett et al. 1994). In 60–70-year-old men, the total bone Pb burden may be ≥200 mg, while children <16 years old have been shown to have a total bone Pb burden of 8 mg (Barry 1975). However, in some bones (i.e., mid femur and pelvic bone), the increase in Pb content plateaus at middle age and then decreases at higher ages; the decrease with age was more pronounced in females (Drasch et al. 1987). Osteoporosis and release of Pb from resorbed bone to blood may contribute to decreasing bone Pb content in females (Gulson et al. 2002).

Evidence for the exchange of bone Pb and soft tissue Pb stores comes from analyses of stable Pb isotope signatures of Pb in bone and blood. A comparison of blood and bone Pb stable isotope signatures in five adults indicated that bone Pb stores contributed to approximately 40–70% of the Pb in blood (Smith et al. 1996). During pregnancy, the mobilization of bone Pb increases, as the bone is resorbed to produce the fetal skeleton. Analysis for kinetics of changes in the stable isotope signatures of blood Pb in pregnant women as they came into equilibrium with a novel environmental Pb isotope signature indicated that 10–88% of the Pb in blood may derive from the mobilization of bone Pb store and approximately 80% of cord blood may be contributed from maternal bone Pb (Gulson 2000; Gulson et al. 1997b, 1999c, 2003). The mobilization of bone Pb during pregnancy may contribute, along with other mechanisms (e.g., increased absorption), to the increase in Pb concentration that has been observed during the later stages of pregnancy (Gulson et al. 1997b, 2016; Lagerkvist et al. 1996; Schuhmacher et al. 1996). Bone resorption during pregnancy can be reduced by ingestion of calcium supplements (Janakiraman et al. 2003). Additional evidence for increased mobilization of bone Pb into blood during pregnancy is provided from studies in nonhuman primates and rats (Franklin et al. 1997; Maldonado-Vega et al. 1996). Direct evidence for transfer of maternal bone Pb to the fetus has been provided from stable Pb isotope studies in Cynomolgus monkeys (*Macaca fascicularis*) that were dosed with Pb having a different stable isotope ratio than the Pb to which the monkeys were exposed at an earlier age; approximately 7–39% of the maternal Pb burden that was transferred to the fetus appeared to have been derived from the maternal skeleton (Franklin et al. 1997).

In addition to pregnancy, other states of increased bone resorption appear to result in release of bone Pb to blood; these include lactation, osteoporosis, and severe weight loss. Analysis of kinetics of changes in the stable isotope signatures of blood Pb in postpartum women as they came into equilibrium with a novel environmental Pb isotope signature indicated that the release of maternal bone Pb to blood appears to accelerate during lactation (Gulson et al. 2002, 2003, 2004). This is consistent with declines in patella bone Pb (measured by XRF) during lactation without calcium supplementation (Henandez-Avila et al.

1996). Similar approaches have detected increased release of bone Pb to blood in women, in association with menopause (Gulson et al. 2002). These observations are consistent with epidemiological studies that have shown increases in PbB after menopause and in association with decreasing bone density in postmenopausal women (Berkowitz et al. 2004; Garrido Latorre et al. 2003; Hernandez-Avila et al. 2000; Korrick et al. 2002; Nash et al. 2004; Popovic et al. 2005; Symanski and Hertz-Picciotto 1995). In a prospective study of women who were scheduled to undergo bilateral oophorectomy for benign conditions, blood and tibia bone Pb (measured by XRF and adjusted for bone mineral density) did not change 6–18 months post-surgery, regardless of whether patients were given estrogen replacement therapy (Berkowitz et al. 2004). Severe weight loss (28% of BMI in 6 months) in women, which increased bone turnover, increased PbB (Riedt et al. 2009).

Pb in Soft Tissues. Several studies have compared soft tissue concentrations of Pb in autopsy samples of soft tissues (Barry 1975, 1981; Gross et al. 1975; Schroeder and Tipton 1968). These studies were conducted in the 1960s and 1970s and, therefore, reflect burdens accrued during periods when ambient and occupational exposure levels were much higher than current levels. A more recent autopsy study found lower Pb concentrations in autopsies performed during the period 2004–2013 (Mari et al. 2014). Average PbBs reported in the adult subjects were approximately 20 μg/dL in the Barry (1975) and Gross et al. (1975) studies, whereas more current estimates of the average for adults in the United States are <5 μg/dL (CDC 2018a). Levels in other soft tissues also appear to have decreased substantially since these studies were reported (Barregård et al. 1999; Mari et al. 2014). For example, average Pb concentrations in kidney cortex of male adults were 0.78 μg/g wet tissue and 0.79 μg/g, as reported by Barry (1975) and Gross et al. (1975), respectively (samples in the Barry study were from subjects who had no known occupational exposures). An analysis of kidney biopsy samples collected in Sweden found that the mean level of lead in kidney cortex among subjects not occupationally exposed to Pb was 0.18 μg/g (maximum, 0.56 μg/g) (Barregård et al. 1999). Mari et al. (2014) reported a value of 0.18 μg/g for mean kidney Pb concentration in 20 autopsies performed in Spain. In spite of the downward trends in soft tissue Pb levels, the autopsy studies provide a basis for describing the relative soft tissue distribution of Pb in adults and children. Most of the Pb in soft tissue is in liver. Relative amounts of Pb in soft tissues as reported by Schroeder and Tipton (1968), expressed as percent of total soft tissue Pb, were: liver, 33%; skeletal muscle, 18%; skin, 16%; dense connective tissue, 11%; fat, 6.4%; kidney, 4%; lung, 4%; aorta, 2%; and brain, 2% (other tissues were <1%). The highest soft tissue concentrations in adults also occur in liver and kidney cortex (Barry 1975; Gerhardsson et al. 1986a, 1995b; Gross et al. 1975; Mari et al. 2014; Oldereid et al. 1993). The relative distribution of Pb in soft tissues, in males and females, expressed in terms of tissue:liver concentration ratios, were: liver, 1.0 (approximately 1 μg/g

wet weight); kidney cortex, 0.8; kidney medulla, 0.5; pancreas, 0.4; ovary, 0.4; spleen, 0.3; prostate, 0.2; adrenal gland, 0.2; brain, 0.1; fat, 0.1; testis, 0.08; heart, 0.07; and skeletal muscle, 0.05 (Barry 1975; Gross et al. 1975). In contrast to Pb in bone, which accumulates Pb with continued exposure in adulthood, concentrations in soft tissues (e.g., liver and kidney) are relatively constant in adults (Barry 1975; Treble and Thompson 1997), reflecting a faster turnover of Pb in soft tissue, relative to bone.

Mechanisms by which Pb enters soft tissues have not been fully characterized (Bressler et al. 2005). Studies conducted in preparations of mammalian small intestine support the existence of saturable and nonsaturable pathways of Pb transfer and suggest that Pb can interact with transport mechanisms for calcium and iron (see Section 3.1.1). Pb can enter cells through voltage-gated L-type Ca^{2+} channels in bovine adrenal medullary cells (Legare et al. 1998; Simons and Pocock 1987; Tomsig and Suszkiw 1991) and through store-operated Ca^{2+} channels in pituitary GH3, glial C3, human embryonic kidney, and bovine brain capillary endothelial cells (Kerper and Hinkle 1997a, 1997b). Anion exchangers may also participate in Pb transport in astrocytes (Bressler et al. 2005). In addition to the small intestine, DMT1 is expressed in the kidney (Canonne-Hergaux et al. 1999); however, little information is available regarding the transport of Pb across the renal tubular epithelium. In Madin-Darby canine kidney cells (MDCK), Pb has been shown to undergo transepithelial transport by a mechanism distinct from the anion exchanger that has been identified in red blood cells (Bannon et al. 2000). The uptake of Pb into MDCK cells was both time and temperature dependent. Overexpression of DMT1 in the human embryonic kidney fibroblast cells (HEK293) resulted in increased Pb uptake compared to HEK293 cells in which DMT1 was not overexpressed (Bannon et al. 2002). Based on this limited information, it appears that DMT1 may play a role in the renal transport of Pb.

Pb in other soft tissues such as kidney, liver, and brain exists predominantly bound to protein. High affinity cytosolic Pb binding proteins have been identified in rat kidney and brain (DuVal and Fowler 1989; Fowler 1989; Gonick et al. 2011). The Pb binding proteins of rat are cleavage products of α2μ-globulin, a member of the protein superfamily known as retinol-binding proteins (Fowler and DuVal 1991). α2μ-Globulin is synthesized in the liver under androgen control and has been implicated in the mechanism of male rat hyaline droplet nephropathy produced by certain hydrocarbons (EPA 1991a; Swenberg et al. 1989); however, there is no evidence that Pb induces male-specific nephropathy or hyaline droplet nephropathy. The precise role for Pb binding proteins in the toxicokinetics and toxicity of Pb has not been firmly established; however, it has been proposed that binding proteins may serve as a cytosolic Pb "receptor" that, when transported into the nucleus, binds to chromatin and modulates gene expression (Fowler and DuVal 1991; Mistry et al. 1985, 1986). Other high-affinity Pb binding proteins

(Kd approximately 14 nM) have been isolated in human kidney, two of which have been identified as a 5 kD peptide, thymosin 4, and a 9 kD peptide, acyl-CoA binding protein (Smith et al. 1998b). Pb also binds to metallothionein, but does not appear to be a significant inducer of the protein in comparison with the inducers of cadmium and zinc (Eaton et al. 1980; Waalkes and Klaassen 1985). *In vivo*, only a small fraction of the Pb in the kidney is bound to metallothionein, and appears to have a binding affinity that is less than Cd^{2+}, but higher than Zn^{2+} (Ulmer and Vallee 1969); thus, Pb will more readily displace zinc from metallothionein than cadmium (Goering and Fowler 1987; Nielson et al. 1985; Waalkes et al. 1984).

Pb Distribution during Pregnancy and Maternal-Fetal-Infant Transfer. PbBs tend to be lower in pregnant women compared to non-pregnant women of similar age, BMI, iron status, and smoking status (Jain 2013a; Liu et al. 2013). This difference may reflect increased elimination of Pb from the maternal system (Jain 2013b). Maternal PbB changes during and following pregnancy. A U-shaped temporal pattern has been observed in which maternal PbBs decrease during the second trimester and increase during the third trimester and postpartum period (Gulson et al. 2004, 1997, 2016; Hertz-Picciotto et al. 2000; Lagerkvist et al. 1996; Lamadrid-Figueroa et al. 2006; Rothenberg et al. 1994a). Several factors appear to contribute to these changes. During the second trimester, increased plasma volume contributes to hemodilution of maternal blood Pb and a lowering in the PbB (Hytten 1985; Rothenberg et al. 1994b). During the third trimester, growth of the fetal skeleton accelerates, which results in increased mobilization of calcium and Pb from the maternal skeleton, increasing maternal PbB (Gulson et al. 1998b, 2003). Postpartum calcium demand increases further during lactation and breastfeeding, which promotes further mobilization of calcium and Pb from bone and sustains or increases maternal PbBs (Gulson et al. 1998b; Hansen 2011; Tellez-Rojo et al. 2002). Increased demand for calcium in the third trimester and postpartum (to supply calcium for breast milk) is also evident from studies of the effects of dietary calcium supplementation during pregnancy. Calcium supplementation of the maternal diet decreased or delayed the onset of the increase in maternal PbB during the third trimester and postpartum period and delayed mobilization of maternal bone Pb in the third trimester (Ettinger et al. 2009; Gulson et al. 2004, 2016; Manton et al. 2003). The increase in PbB associated with late pregnancy was greater in older women who had a longer history of Pb exposure and, presumably, higher bone Pb levels (Miranda et al. 2010). Pb has been detected in follicular fluid at concentrations similar to that in blood plasma (Silberstein et al. 2006).

A portion of the maternal Pb burden is transferred to the placenta and fetus during pregnancy (Esteban-Vasaloo et al. 2012; Franklin et al. 1997; Gulson et al. 2003, 2016; Kayaalti et al. 2016; Kazi et al. 2014; O'Flaherty 1998; Reddy et al. 2014). Measurements of stable Pb isotope ratios in pregnant women and

cord blood, as they came into equilibrium with a novel environmental Pb isotope signature, indicated that approximately 80% of Pb in fetal cord blood appears to derive from maternal bone stores (Gulson et al. 1997b, 1999c, 2000, 2003, 2016). Stable isotope studies have also demonstrated transfer of Pb from the maternal skeleton to fetus in nonhuman primates (Franklin et al. 1997; O'Flaherty 1998). Transplacental transfer of Pb may be facilitated by an increase in the plasma/PbB ratio during pregnancy (Lamadrid-Figueroa et al. 2006; Montenegro et al. 2008).

Fetal and maternal PbBs and placental Pb concentrations are correlated (Amaral et al. 2010; Baeyens et al. 2014; Baranowska-Boisiacka et al. 2016; Carbone et al. 1998; Chen et al. 2014; Goyer 1990; Graziano et al. 1990; Gulson et al. 2016; Kayaalti et al. 2015b; Kazi et al. 2014; Kim et al. 2015; Kordas et al. 2009; Patel and Prabhu 2009; Reddy et al. 2014). Estimates of the maternal/fetal PbB ratio, based on cord blood Pb measurements at the time of delivery, range from 0.7 to 1.0 at mean maternal PbBs ranging from 1 to 9 μg/dL. In one of the larger studies of fetal PbB, maternal and cord PbB were measured at delivery in 888 mother-infant pairs; the cord/maternal ratio was relatively constant, 0.93, over a blood Pb range of approximately 3–40 μg/dL (Graziano et al. 1990). An analysis of data from 159 mother-infant pairs revealed that higher blood pressure and alcohol consumption late in pregnancy were associated with higher concentrations of Pb in cord blood relative to maternal blood, while higher hemoglobin and sickle cell trait were associated with lower cord blood Pb relative to maternal blood Pb (Harville et al. 2005). No associations were found for calcium intake, physical activity, or smoking. Placental Pb concentrations were found to correlate with ALAD polymorphisms, with higher concentrations observed in association with ALAD2 (Kayaalti et al. 2015b).

Maternal Pb is transferred to infants during breastfeeding. Stable Pb isotope dilution studies suggested that Pb in breast milk can contribute substantially to the isotope profile of infant blood (approximately 40–80%; Gulson et al. 1998b). Numerous studies have reported Pb concentrations in maternal blood and breast milk. In general, these studies indicate that Pb concentrations in breast milk are correlated with Pb concentrations in maternal blood or plasma. Milk/maternal concentration ratios are <0.1, although values of 0.9 have been reported (Baranowska-Boisiacka et al. 2016; Counter et al. 2014; Ettinger et al. 2006, 2014; Gulson et al. 1998a; Koyashiki et al. 2010). Ettinger et al. (2004, 2006) assessed factors influencing breast milk Pb concentration in a group of 367 women and found that PbB (mean 8–9 μg/dL; range 2–30) was a stronger predictor of breast milk Pb (mean 0.9–1.4 μg/dL; range 0.2–8 μg/dL) than bone Pb, and that tibia Pb (mean 9.5 μg/g; range <1–76.5 μg/dL) was a stronger predictor of breast milk Pb than patella bone Pb (mean 14.6 μg/dL; range <1–67.2 μg/dL). Dietary intake of polyunsaturated fatty acids (PUFA) may decrease transfer of Pb from bone to breast milk (Arora et al. 2008). Pb

concentrations in maternal blood and breast milk have been shown to correlate with PbBs in breastfeeding infants (Ettinger et al. 2014; Farhat et al. 2013). Breast milk Pb concentrations explained 37% of the variation in infant blood Pb of breastfeeding infants (Ettinger et al. 2014).

Organic Pb. Information on the distribution of Pb in humans following exposures to organic Pb is extremely limited. One hour following 1–2-minute inhalation exposures to ^{203}Pb tetraethyl or tetramethyl Pb (1 mg/m^3), approximately 50% of the ^{203}Pb body burden was associated with liver and 5% was associated with kidney; the remaining ^{203}Pb was widely distributed throughout the body (Heard et al. 1979). The kinetics of ^{203}Pb in blood of these subjects showed an initial declining phase during the first 4 hours (tetramethyl Pb) or 10 hours (tetraethyl Pb) after the exposure, followed by a phase of gradual increase in PbB that lasted for up to 500 hours after the exposure. Radioactive Pb in blood was highly volatile immediately after the exposure and transitioned to a nonvolatile state thereafter. These observations may reflect an early distribution of organic Pb from the respiratory tract, followed by a redistribution of de-alkylated Pb compounds (see Section 3.1.3 for further discussion of alkyl Pb metabolism).

In a man and woman who accidentally inhaled a solvent containing 31% tetraethyl Pb (17.6% Pb by weight), Pb concentrations in the tissues, from highest to lowest, were liver, kidney, brain, pancreas, muscle, and heart (Bolanowska et al. 1967). In another incident, a man ingested a chemical containing 59% tetraethyl Pb (38% Pb w/w); Pb concentration was highest in the liver followed by kidney, pancreas, brain, and heart (Bolanowska et al. 1967).

3.1.3 Metabolism

Inorganic Pb. Metabolism of inorganic Pb consists of formation of complexes with a variety of protein and nonprotein ligands (see Section 3.1.2 for further discussion). Major extracellular ligands include albumen and nonprotein sulfhydryls. The major intracellular ligand in red blood cells is ALAD. Pb also forms complexes with proteins in the cell nucleus and cytosol.

Organic Pb. Alkyl Pb compounds are actively metabolized in the liver by oxidative dealkylation catalyzed by cytochrome P-450. Relatively few studies that address the metabolism of alkyl Pb compounds in humans have been reported. Studies of workers who were exposed to tetraethyl Pb have shown that tetraethyl Pb is excreted in the urine as diethyl Pb, ethyl Pb, and inorganic Pb (Turlakiewicz and Chmielnicka 1985; Vural and Duydu 1995; Zhang et al. 1994). Trialkyl Pb metabolites were found

in the liver, kidney, and brain following exposure to the tetraalkyl compounds in workers; these metabolites have also been detected in brain tissue of nonoccupational subjects (Bolanowska et al. 1967; Nielsen et al. 1978). In volunteers exposed by inhalation to 0.64 and 0.78 mg Pb/m^3 of ^{203}Pb-labeled tetraethyl and tetramethyl Pb, respectively, Pb was cleared from the blood within 10 hours, followed by a re-appearance of radioactivity back into the blood after approximately 20 hours (Heard et al. 1979). The high level of radioactivity initially in the plasma indicates the presence of tetraalkyl/trialkyl Pb. The subsequent rise in blood radioactivity, however, probably represents water-soluble inorganic Pb and trialkyl and dialkyl Pb compounds that were formed from the metabolic conversion of the volatile parent compounds (Heard et al. 1979).

3.1.4 Excretion

Independent of the route of exposure, absorbed Pb is excreted primarily in urine and feces; sweat, saliva, hair and nails, breast milk, and seminal fluids are minor routes of excretion (Chamberlain et al. 1978; Griffin et al. 1975; Hernandez-Ochoa et al. 2005; Hursh and Suomela 1968; Hursh et al. 1969; Kehoe 1987; Rabinowitz et al. 1976; Sears et al. 2012; Stauber et al. 1994). Fecal excretion accounts for approximately one-third of total excretion of absorbed Pb (fecal/urinary excretion ratio of approximately 0.5), based on intravenous injection studies conducted in humans (Chamberlain et al. 1978). A similar value for fecal/urinary excretion ratio, approximately 0.5, has been observed following inhalation of submicron Pb particles (Chamberlain et al. 1978; Hursh et al. 1969). Contributors to fecal excretion may include secretion into the bile, gastric fluid, and saliva (Rabinowitz et al. 1976). Biliary excretion of Pb has been observed in the dog, rat, and rabbit (Klaassen and Shoeman 1974; O'Flaherty 1993).

Mechanisms by which inorganic Pb is excreted in urine have not been fully characterized. Such studies have been hampered by the difficulties associated with measuring ultrafilterable Pb in plasma and thereby in measuring the GFR of Pb. Renal plasma clearance was approximately 20–30 mL/minute in a subject who received a single intravenous injection of a ^{203}Pb chloride tracer (Chamberlain et al. 1978). Urinary Pb excretion is strongly correlated with the GFR of Pb (Araki et al. 1986) and plasma Pb concentration (Bergdahl et al. 1997b; Rentschler et al. 2012) (i.e., urinary excretion is proportional to GFR x plasma Pb concentration). Estimates of plasma-to-urine clearance of Pb range from 13 to 22 L/day, with a mean of 18 L/day (Araki et al. 1986; Manton and Cook 1984; Manton and Malloy 1983; Chamberlain et al. 1978). The rate of urinary excretion of Pb was less than the GFR of ultrafilterable Pb, suggesting renal tubular reabsorption of Pb from the glomerular filtrate (Araki et al. 1986, 1990). Measurement of the renal clearance of ultrafilterable Pb in plasma indicates that in dogs, Pb undergoes glomerular filtration and net

tubular reabsorption (Araki et al. 1986, 1990; Vander et al. 1977; Victery et al. 1979). Net tubular secretion of Pb has been demonstrated in dogs made alkalotic by infusions of bicarbonate (Victery et al. 1979). Renal clearance of blood Pb increases with increasing PbBs >25 µg/dL (Chamberlain 1983). The mechanism for this has not been elucidated and could involve a shift in the distribution of Pb in blood towards a fraction having a higher GFR (e.g., lower molecular weight complex), a capacity-limited mechanism in the tubular reabsorption of Pb, or the effects of Pb-induced nephrotoxicity on Pb reabsorption.

Excretion and Routes of Exposure

Inhalation Exposure

Inorganic Pb. Inorganic Pb inhaled as submicron particles is deposited primarily in the bronchiolar and alveolar regions of the respiratory tract, from where it is absorbed and excreted primarily in urine and feces (Chamberlain et al. 1978; Hursh et al. 1969; Kehoe 1987). Fecal/urinary excretion ratios were approximately 0.5 following inhalation of submicron Pb-bearing particles (Chamberlain et al. 1978; Hursh et al. 1969). Higher fecal-urinary ratios would be expected following inhalation of larger particle sizes (e.g., >1 µm) as these particles would be cleared to the gastrointestinal tract from where a smaller percentage would be absorbed (Kehoe 1987; see Section 3.1.1).

Organic Pb. Pb derived from inhaled tetraethyl and tetramethyl Pb is excreted in exhaled air, urine, and feces (Heard et al. 1979). Following 1–2-minute inhalation exposures to ^{203}Pb tetraethyl (1 mg/m^3), in four male subjects, 37% of inhaled ^{203}Pb was initially deposited in the respiratory tract, of which approximately 20% was exhaled in the subsequent 48 hours (Heard et al. 1979). In a similar experiment conducted with (^{203}Pb) tetramethyl Pb, 51% of the inhaled ^{203}Pb dose was initially deposited in the respiratory tract, of which approximately 40% was exhaled in 48 hours. Pb that was not exhaled was excreted in urine and feces. Fecal/urinary excretion ratios were 1.8 following exposure to tetraethyl Pb and 1.0 following exposure to tetramethyl Pb (Heard et al. 1979). Occupational monitoring studies of workers who were exposed to tetraethyl Pb have shown that tetraethyl Pb is excreted in the urine as diethyl Pb, ethyl Pb, and inorganic Pb (Turlakiewicz and Chmielnicka 1985; Vural and Duydu 1995; Zhang et al. 1994).

Oral Exposure

Inorganic Pb. Much of the available information on the excretion of ingested Pb in adults derives from studies conducted on five male adults who received daily doses of ^{207}Pb nitrate for periods up to 210 days (Rabinowitz et al. 1976). The dietary intakes of the subjects were reduced to accommodate the tracer doses of ^{207}Pb without increasing daily intake, thus preserving a steady state with respect to total Pb intake and excretion. Total Pb intakes (diet plus tracer) ranged from approximately 210 to 360 µg/day. Urinary excretion accounted for approximately 12% of the daily intake (range for five subjects: 7–17%) and fecal excretion, approximately 90% of the daily intake (range, 87–94%). Based on measurements of tracer and total Pb in saliva, gastric secretions, bile, and pancreatic secretions (samples collected from three subjects by intubation), gastrointestinal secretion of Pb was estimated to be approximately 2.4% of intake (range, 1.9–3.3%). In studies conducted at higher ingestion intakes, 1–3 mg/day for up to 208 weeks, urinary Pb excretion accounted for approximately 5% of the ingested dose (Kehoe 1987).

Dermal Exposure. Inorganic Pb is excreted in sweat and urine following dermal exposure to Pb nitrate or Pb acetate (Moore et al. 1980; Stauber et al. 1994).

3.1.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

PBPK models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic endpoints.

Early Pb modeling applications relied on classical pharmacokinetics. Compartments representing individual organs or groups of organs that share a common characteristic were defined as volumes, or pools, that are kinetically homogeneous. For example, the body could be represented by a central compartment (e.g., blood plasma), and one or two peripheral compartments, which might be "shallow" or "deep" (i.e., they may exchange relatively rapidly or relatively slowly with blood plasma) (O'Flaherty 1987). One of the first of such models was proposed by Rabinowitz et al. (1976) based on a study of the

kinetics of ingested stable Pb isotope tracers and Pb balance data in five healthy adult males. The Rabinowitz model included three compartments: a central compartment representing blood and other tissues and spaces in rapid equilibrium with blood (e.g., interstitial fluid); a shallow tissue compartment, representing soft tissues and rapidly exchanging pools within the skeleton; and a deep tissue compartment, representing, primarily, slowly exchanging pools of Pb within bone. Excretion pathways represented in the model included urinary, from the central compartment, and bile, sweat, hair, and nails, from the shallow tissue compartment. The model predicted pseudo-first-order half-times for Pb of approximately 25, 28, and 10^4 days in the central, shallow tissue, and deep compartments, respectively. The slow kinetics of the deep tissue compartment leads to the prediction that it would contain most of the Pb burden after lengthy exposures (e.g., years), consistent with Pb measurements made in human autopsy samples (see Section 3.1.2 Distribution). Note that this model did not simulate the distribution of Pb within blood (e.g., erythrocytes and plasma), nor did it simulate subcompartments within bone or physiological processes of bone turnover that might affect kinetics of the deep tissue compartment.

Marcus (1985b) reanalyzed the data from stable isotope tracer studies of Rabinowitz et al. (1976) and derived an expanded multicompartment kinetic model for Pb that included separate compartments for cortical (slow, $t_{1/2}$ $1.2x10^4$–$3.5x10^4$ days) and trabecular (fast, $t_{1/2}$ 100–700 days), an approach subsequently adopted in several models (Bert et al. 1989; EPA 1994a, 1994b; Leggett 1993; O'Flaherty 1993, 1995a). A more complex representation of the Pb disposition in bone included explicit simulation of diffusion of Pb within the bone volume of the osteon and exchange with blood at the canaliculus (Marcus 1985a). The bone diffusion model was based on Pb kinetics data from studies conducted in dogs. Marcus (1985c) also introduced nonlinear kinetics of exchange of Pb between plasma and erythrocytes. The blood model included four blood subcompartments: diffusible Pb in plasma, protein-bound Pb in plasma, a "shallow" erythrocyte pool, and a "deep" erythrocyte pool. This model predicted the curvilinear relationship between plasma and PbBs observed in humans (see Section 3.1.2 Distribution for further discussion of plasma-erythrocyte Pb concentrations).

Additional information on Pb biokinetics, bone mineral metabolism, and Pb exposures has led to further refinements and expansions of these earlier modeling efforts. Four pharmacokinetic models, in particular, are currently being used or are being considered for broad application in Pb risk assessment: (1) the O'Flaherty Model, which simulates Pb kinetics from birth through adulthood (O'Flaherty 1993, 1995a); (2) the EPA Integrated Exposure Uptake BioKinetic (IEUBK) Model for Lead in Children developed by EPA (1994a, 1994b); (3) the Leggett Model, which simulates Pb kinetics from birth through adulthood

(Leggett 1993); and (4) the EPA All Ages Lead Model (AALM, EPA 2014a). The structure and parameterization of the O'Flaherty Model is distinct from both the IEUBK Model and Leggett Model. The AALM is an update of the O'Flaherty and Leggett models, extended to include a multi-media exposure model.

The IEUBK Model simulates multimedia exposures, uptake, and kinetics of Pb in children ages 0–7 years for predicting pseudo-steady state relationships between Pb exposure and PbB; the model is not intended for use in predicting short-term kinetics of blood Pb or Pb concentrations in tissues other than whole blood. The O'Flaherty Model, Leggett Model, and AALM are lifetime models, and include parameters that simulate uptake and kinetics of Pb during infancy, childhood, adolescence, and adulthood. Pb exposure (e.g., residence-specific environmental Pb concentrations, childhood activity patterns) is not readily described by current versions of the O'Flaherty and Leggett models. The IEUBK Model and AALM include parameters for simulating exposures and uptake to estimate average daily uptake of Pb (μg/day) among populations potentially exposed via soil and dust ingestion, air inhalation, tap water ingestion, diet, and miscellaneous (other) intakes. All four models have been calibrated, to varying degrees, against empirical physiological data on animals and humans, and data on PbBs in individuals and/or populations (Beck et al. 2001; Bowers and Mattuck 2001; Cal EPA 2013; EPA 1994a, 1994c, 2014a, 2014b, 2016; Griffin et al. 1999; Hogan et al. 1998; Leggett 1993; Li et al. 2016; MacMillan et al. 2015; O'Flaherty 1993, 1995, 1998, 2000; Pounds and Leggett 1998; White et al. 1998; Von Lindern et al. 2003, 2016).

The focus on relying on PbBs for model evaluation and calibration derives from several concerns. The empirical basis for a relationship between low levels of Pb exposure and behavioral dysfunction largely consists of prospective epidemiological studies relating various indices of dysfunction with PbB (see Section 3.3). In this context, PbB has been related to health effects of Pb, and this is the main reason that the focus of interest in the models has been on estimating PbBs. Also, the most available data with which to calibrate and validate the models have been data relating exposure and/or Pb intake to blood concentration. Thus, there is greater confidence in the validity of the models for estimating blood concentrations, rather than Pb levels in other physiologic compartments. Although the principal adverse health effects of Pb have been related to concentrations of Pb in blood, other biomarkers of Pb exposure, such as bone Pb concentrations, are also of value in assessing associations between Pb exposure and health; hence, there is a need for models that predict concentrations of Pb in tissues other than blood (see Section 3.3).

The following four pharmacokinetic models are discussed in great detail below: (1) the O'Flaherty Model (O'Flaherty 1993, 1995a); (2) the IEUBK Model for Lead in Children (EPA 1994a, 1994b); (3) the Leggett Model (Leggett 1993); and (4) AALM (EPA 2014a, 2016).

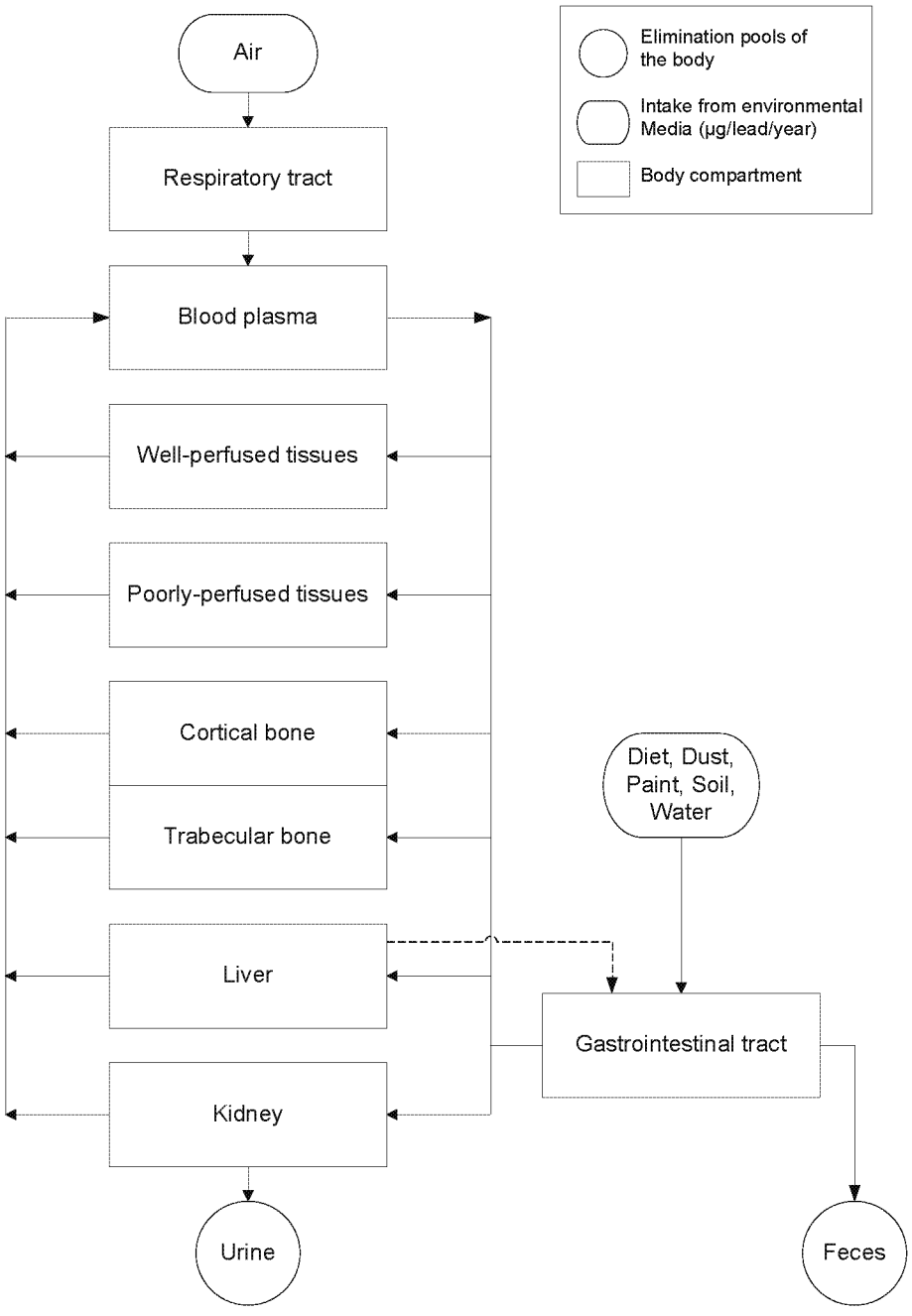
3.1.5.1 O'Flaherty Model

The O'Flaherty Model simulates Pb exposure, uptake, and disposition in humans, from birth through adulthood (O'Flaherty 1993, 1995a). Figure 3-1 shows a conceptualized representation of the O'Flaherty Model, including the movement of Pb from exposure media (i.e., intake via inhalation or ingestion) to the lungs and gastrointestinal tract, followed by the subsequent exchanges between blood plasma, liver, kidney, richly-perfused tissues, poorly-perfused tissues, bone compartments, and excretion from liver and/or kidney. The model simulates both age- and media-specific absorption. Because many of the pharmacokinetic functions are based on body weight and age, the model can be used to estimate PbBs across a broad age range, including infants, children, adolescents, and adults. The model uses physiologically based parameters to describe the volume, composition, and metabolic activity of blood, soft tissues, and bone that determine the disposition of Pb in the human body.

A central feature of the model is the growth curve, a logistic expression relating body weight to age. The full expression relating weight to age has five parameters (constants), so that it can readily be adapted to fit a range of standardized growth curves for men and women. Tissue growth and volumes are linked to body weight; this provides explicit modeling of concentrations of Pb in tissues. Other physiologic functions (e.g., bone formation) are linked to body weight, age, or both.

Pb exchange between blood plasma and bone is simulated as parallel processes occurring in cortical (80% of bone volume) and trabecular bone (20% of bone volume). Uptake and release of Pb from trabecular bone and metabolically active cortical bone are functions of bone formation and resorption rates, respectively. Rates of bone formation and resorption are simulated as age-dependent functions, which gives rise to an age-dependence of Pb kinetics in bone. The model simulates an age-related transition from immature bone, in which bone turnover (formation and resorption) rates are relatively high, to mature bone, in which turnover is relatively slow. Changes in bone mineral turnover associated with senescence (e.g., postmenopausal osteoporosis) are not represented in the model. In addition to metabolically active regions of bone, in which Pb uptake and loss is dominated by bone formation and loss, a region of slow kinetics in mature cortical bone is also simulated, in which Pb uptake and release to blood occur by heteroionic exchange with other minerals (e.g., calcium). Heteroionic exchange is

Figure 3-1. Compartments and Pathways of Lead (Pb) Exchange in the O'Flaherty Model*



*Schematic model for Pb kinetics in which Pb distribution is represented by flows from blood plasma to liver, kidney, richly-perfused tissues, poorly-perfused tissues, and cortical and trabecular bone. The model simulates tissue growth with age, including growth and resorption of bone mineral.

Sources: O'Flaherty 1991b, 1993, 1995a

simulated as a radial diffusion in bone volume of the osteon. All three processes are linked to body weight, or the rate of change of weight with age. This approach allows for explicit simulation of the effects of bone formation (e.g., growth) and loss, changes in bone volume, and bone maturation on Pb uptake and release from bone. Exchanges of Pb between blood plasma and soft tissues (e.g., kidney and liver) are represented as flow-limited processes. The model simulates saturable binding of Pb in erythrocytes; this replicates the curvilinear relationship between plasma and erythrocyte Pb concentrations observed in humans (see Section 3.1.2). Excretory routes include kidney to urine and liver to bile. Total excretion (clearance from plasma attributable to bile and urine) is simulated as a function of GFR. Biliary and urinary excretory rates are proportioned as 70 and 30% of the total plasma clearance, respectively.

The O'Flaherty Model simulates Pb intake from inhalation and ingestion. Inhalation rates are age-dependent. Absorption of inhaled Pb is simulated as a fraction (0.5) of the amount inhaled, and is independent of age. The model simulates ingestion exposures from infant formula, soil and dust ingestion, and drinking water ingestion. Rates of soil and dust ingestion are age-dependent, increasing to approximately 130 mg/day at age 2 years, and declining to <1 mg/day after age 10 years. Gastrointestinal absorption of Pb in diet and drinking water is simulated as an age-dependent fraction, declining from 0.58 of the ingestion rate at birth to 0.08 after age 8 years. These values can be factored to account for relative bioavailability when applied to absorption of Pb ingested in dust or soil.

The O'Flaherty Model, as described in O'Flaherty (1993, 1995a), utilizes point estimates for parameter values and yields point estimates as output; however, a subsequent elaboration of the model has been developed that utilizes a Monte Carlo approach to simulate variability in exposure, absorption, and erythrocyte Pb binding capacity (Beck et al. 2001). This extension of the model can be used to predict the probability that children exposed to Pb in environmental media will have PbBs exceeding a health-based level of concern (e.g., 5 μg/dL).

The model was designed to operate with an exposure time step on 1 year (the smallest time interval for a single exposure event). However, the implementation code allows constructions of simulations with an exposure time step as small as 1 day, which would allow simulation of rapidly changing intermittent exposures (e.g., an acute exposure event).

The O'Flaherty Model was initially calibrated to predict blood, bone, and tissue Pb concentrations in rats (O'Flaherty 1991a), and subsequently modified to reflect anatomical and physiological characteristics in